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Allogona profunda (Say), a colorful native land snail species found in central and eastern Kentucky (see Dourson, page 119, this issue).

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A Selected Land Snail Compilation of the Central Knobstone Escarpment on Furnace Mountain in Powell County Kentucky

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ABSTRACT

The land snail fauna of Furnace Mountain, Powell County, Kentucky is reported. Fifteen families, 35 genera, and 61 native species were documented, containing nearly all the regionally available fauna from an area encompassing 2 ha. Furnace Mountain is a diverse molluscan refugia in Kentucky, representing the most speciose locale reported in North America and is of global importance for land snail biodiversity. The land snail fauna of Furnace Mountain slightly exceeds New Zealand's Waipipi Scenic Reserve, reported as the richest land snail fauna in the world. Species richness was correlated with the area's extensive Newman limestone outcroppings, calcareous soils and its geographic position on the Central Knobstone Escarpment. Reported here as a "geophysical landscape edge", the area is an amalgamation of the Cumberland Plateau, the Knobs, and the Outer Bluegrass region of Kentucky. The convergence of these three diverse snail eco-regions has provided a number of land snail species the opportunities to coexist.

KEY WORDS: Furnace Mountain, land snails, Central Knobstone Escarpment

INTRODUCTION

Furnace Mountain was selected for a two year study to document the diversity of land snails occupying a mesic, north-facing hillside positioned on the Central Knobstone Escarpment. Locally referred to as Furnace Mountain (Powell County, Kentucky), the area is more correctly an escarpment where three physiographic regions meet, the Cumberland Plateau, the Knobs, and the Outer Bluegrass of Kentucky. The merging of these distinct regions was expected to have exceptional diversity of land snails.

Nearly world-wide in distribution, land snail communities are reported from sub-Antarctic islands harboring only one species to tropical rainforests containing more than fifty taxa (Solem 1984; Emberton 1995). Although Solem and Climo (1985) reported that snail faunas rarely exceed a dozen species, recent studies suggest higher rates of sympatry. Tropical forests, in general, have reported the highest snail faunas. A site near Manombo, Madagascar harbored 52 species from a 4 ha site (Emberton 1995), 50 species were reported from a <4 ha site near Amboni Cave in eastern Tanzania (Emberton et al. 1997), and 45 species were collected from a 400 m^2 area in southwestern Cameroon, Africa (de Winter and Gittenberger 1998). Temperate regions also can hold high species diversity. Eighty-one species were reported from Mammoth Cave National Park (Hubricht 1986), 86 species from the Hiawassee River Basin in Tennessee (Coney et al. 1982), and 44 species from an approximately 4 ha site on Pine Mountain, Kentucky (Hubricht et al. 1983).

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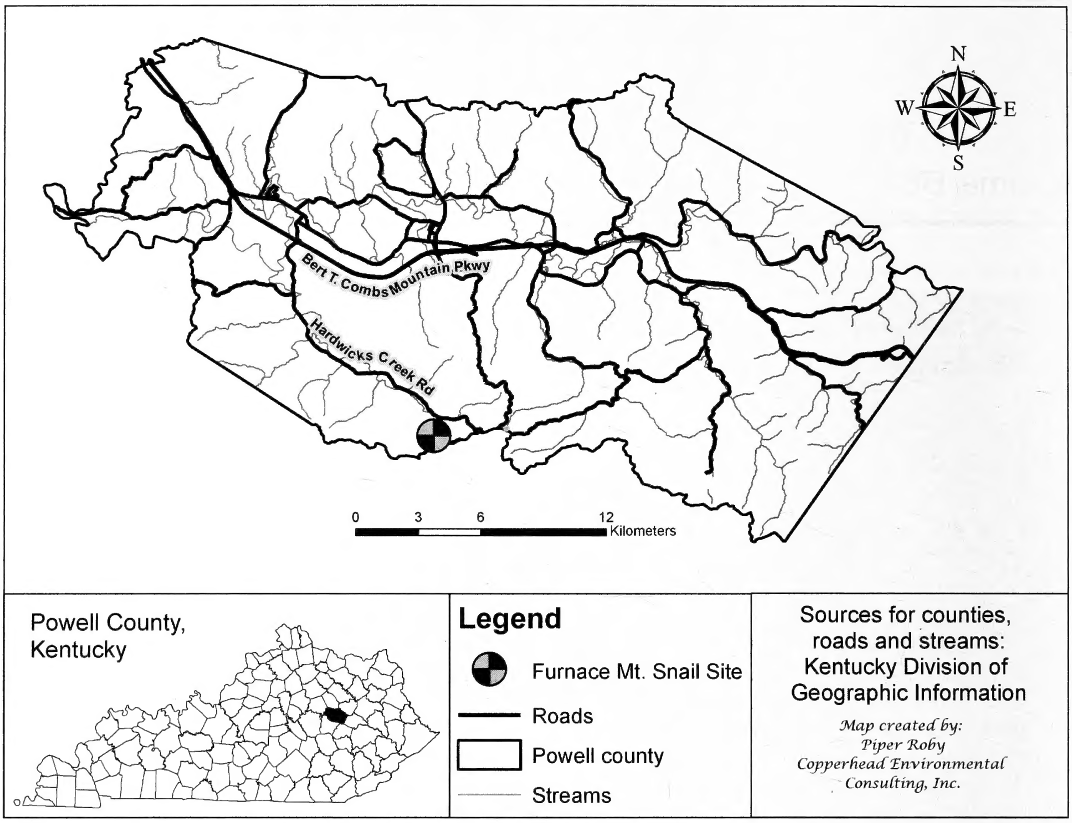

Figure 1. Furnace Mountain study area located along the Central Knobstone Escarpment in eastern Kentucky.

STUDY AREA

Taylor et al. (1997) placed the study area (Figure 1) within the Northern Cumberland Plateau section. The Northern Cumberland Plateau area was first described by McNab and Avers (1994). Taylor et al. (1997) further refined portions of the Northern Cumberland Plateau dividing it into land type associations. The Central Knobstone Escarpment (CKE) land type association is located in east central Kentucky in portions of Bath, Estill, Jackson, Madison, Menifee, Montgomery, and Powell counties. The Furnace Mountain study is located in southern Powell County. This land type association is characterized by extensive dissection, narrow to moderately broad ridges, and narrow valleys with moderate relief and elevation on surfaces of flat-bedded Mississippian and Pennsylvanian aged rocks. Cliffs are common, well-developed, and sometimes prominent. Karst features are moderately well-developed in some areas. They frequently are demarcated from side slopes by low to high cliffs and often have knob-like appearances. Some ridges are pinched off forming cliff-bound knobs. As a unit, the ridges and knobs often are called the Knobstone escarpment, i.e., knob forms, but with well-developed cliffs (McFarland 1943; Fenneman 1938). Side slopes are usually over 40% slope and often exceed 64%. Soils are deep to moderately deep, grayish brown Ultisols, reddish Alfisols, and more shallow Inceptisols. Geologic structure is that of bedded and interbedded sedimentary rocks with a gentle dip to the southeast. Within this land type association, the Appalachian oak forest type and its variants are dominant. A mixed mesophytic forest component is found along streams in deep valleys and on cool, north-facing slopes (Taylor et al. 1997).

Woods et al. (2003) placed this area within the Knobs-Lower Scioto Dissected Plateau in a transition area between the Outer Bluegrass and the Northern Forested Plateau Escarpment. The area is described as high in

Figure 2. Photograph of a 2 ha site located at Furnace Mountain in Powell County, Kentucky. This limestone cliff found along the Central Knobstone Escarpment typifies an area where land snail species diversity can reach exceptional numbers.

topographic and geologic variation, which in turn creates substantial ecological diversity. Woods et al. (2003) agreed with Taylor et al. (1997) that this area is not part of the Interior Plateau (Bluegrass and related area). They, unlike Taylor et al. (1997), placed it in the Western Allegheny Plateau eco-region.

The land snail compilation of Furnace Mountain included a linear 2 ha forested habitat at 366 m that lay directly below and above a limestone cliff-line that averaged about 6–9 m high (Figure 2). Environments below the cliff-line were mixed mesophytic and contained mature white oak *Quercus alba* Linnaeus, sugar maple *Acer saccharum* Marshall, American beech *Fagus grandifolia* Ehrhart, and flowering dogwood *Cornus florida* Weston. Herbaceous ground cover was abundant containing numerous species of plants including red trillium *Trillium erectum* Linnaeus, large-flowered trillium *Trillium grandiflorum* Michaux, yellow lady's slipper *Cypripedium pubescens* Correll, dwarf larkspur *Delphinium tricorne* Michaux, jack-in-the pulpit *Arisaema triphyllum* Schott, white baneberry *Actaea pachypoda* Elliott, bloodroot *Sanguinaria canadensis* Linnaeus, twinleaf *Jeffersonia diphylla* Bartram, and ginseng *Panax quinquefolium* Linneaus.

Micro-habitats such as fallen and rotting hardwood trees in advanced stages of decay and patches of deep leaf litter were found in

relative abundance below the cliff-line. Small well- scattered screes were situated at the base of or down slope of the limestone wall. The cliff-line outcrops were generally dry and did not provide much leaf cover, and few snails were found. Above the cliffs, conditions were more xeric and forest cover was sparsely represented by eastern red cedar *Juniperus virginiana* Linneaus, post oak *Quercus stellata* Wangenheim, and blue ash *Fraxinus quadrangulata* Michaux. Large, dry limestone outcrops occurred here providing potential habitat for Pupillidae species. A small, 1 m^2 ephemeral pool provided potential habitat for wetland-adapted snails.

METHODS

No attempts were made to randomize searches. Instead, collections were based on finding as many variants in microhabitats and their associated land snails occurring across the 2 ha plot. Shells of snails were hand-picked from under leaf litter, rocks, logs, and the surfaces of exposed limestone cliffs. Slugs were collected from under the exfoliating bark of standing dead trees and rotting hardwood logs in advanced stages of decay. Several snail species were taken from around the small ephemeral pool located on top of the limestone cliff.

To best represent the micro-snail fauna of Furnace Mountain, 25, 30 cm^2 selected soil/leaf samples were collected. The samples were thoroughly dried and then sifted through a series of sieves ranging from 4.76–0.50 mm. Snails were removed and sorted. All recovered and identifiable shells were assigned to species using Pilsbry (1940, 1946, 1948), Burch (1962), the author's reference collections and various other recent publications. Taxonomy follows Turgeon et al. (1998).

New county records were determined using Hubricht (1985) and Branson (1973). A sample collection was sent for verification to John Slapcinsky at the Field Museum in Chicago, Illinois, where it is currently being housed.

RESULTS

Approximately 1200 specimens were collected representing 15 families, 35 genera, and 61 species (Table 1). Of these, 33 had not been reported from Powell County by Branson (1973) or by Hubricht (1985). Although the snail fauna was largely comprised of species that were considered common and widely distributed in Kentucky, there were several noteworthy snails. *Anguispira kochi* (Pfieffer) (Figure 3), a common snail at Furnace Mountain, is currently on the Kentucky State Nature Preserves Commission's unpublished watch list (Laudermilk, Kentucky Nature Preserves Commission, pers. com.). Although many sites have been documented throughout the Bluegrass Region in Kentucky and westward, extant populations of *A. kochi* are rare (MacGregor, Kentucky Department of Fish and Wildlife Resources, pers. com.). Live adults and juvenile specimens of *A. kochi* ranging in size from 8–28 mm were found, all below and within 50 m of the cliff-line.

Interestingly, snails from both the Cumberland Plateau and Bluegrass of Kentucky were found to co-exist here. *Mesomphix vulgatus* H. B. Baker (Figure 4), a snail largely associated with Lexington limestone of low hills and ravines in the Bluegrass, occurred at Furnace Mountain at the base of Newman limestone near the summit escarpment of the Cumberland Plateau. Documentation of *M. vulgatus* at Furnace Mountain puts this species at its eastern limits of distribution in the state (Hubricht 1985; Branson 1973). The southern Appalachian endemic *Glyphyalinia cumberlandiana* (Clapp) was a common species on Furnace Mountain, but it does not occur west of this region in Kentucky (Hubricht 1985).

Prior to this survey, *Vallonia perspectiva* Sterki, was known only from Mercer County. It is a holarctic species of waste places (Hubricht 1985), but at Furnace Mountain it was found in dry detritus around eastern red cedar trees. *Punctum vitreum* H. B. Baker, a little known and often misidentified snail, had been reported from only two counties in Kentucky (Branson 1973). The species is likely more widespread than Branson and Hubricht reported. A fourth species of interest found at Furnace Mountain was *Paravitrea petrophila* Bland, previously known only from Pulaski County, Kentucky (Hubricht 1985). No non-native land snails or slugs were reported.

Two typically frequent Central Knobstone Escarpment (CKE) snails, *Patera appressa* (Say) (Figure 5) and *Triodopsis complanata*

Table 1. Land snail species documented at Furnace Mountain in Powell County, Kentucky.

Family	Genus Species	County Record
POMATIOPSIDAE	*Pomatiopsis lapidaria* (Say, 1817)	*
CARYCHIIDAE	*Carychium clappi* Hubricht, 1959	*
	Carychium exile I. Lea, 1842	*
	Carychium nannodes Clapp, 1905	*
COCHLICOPIDAE	*Cochlicopa morseana* (Doherty, 1878)	
VALLONIIDAE	*Vallonia perspective* Sterki, 1892	*
	Vallonia excentrica Sterki, 1893	*
PUPILLIDAE	*Gastrocopta armifera* (Say, 1821)	*
	Gastrocopta contracta (Say, 1822)	*
	Gastrocopta corticaria (Say, 1816)	*
	Gastrocopta pentadon (Say, 1821)	*
	Pupoides albilabris (C. B. Adams, 1821)	*
	Vertigo gouldi (A. Binney, 1823)	*
STROBILOPSIDAE	*Strobilops aenea* Pilsbry, 1926	
	Strobilops labrythinca (Say, 1817)	*
HAPLOTREMATIDAE	*Haplotrema concavum* (Say, 1821)	
PUNCTIDAE	*Punctum minutissimum* (I. Lea, 1841)	*
	Punctum vitreum H. B. Baker, 1930	*
HELICODISCIDAE	*Helicodiscus notius* Hubricht, 1962	*
DISCIDAE	*Anguispira mordax* (Shuttleworth, 1852)	*
	Anguispira kochi (Pfieffer, 1845)	*
	Anguispira alternata (Say, 1816)	
	Discus patulus (Deshayes, 1830)	
PHILOMYCIDAE	*Philomycus carolinianus* (Bosc, 1802)	
	Philomycus flexuolaris Rafinesque, 1820	
	Philomycus togatus (Gould, 1841)	*
	Pallifera dorsalis (A. Binney, 1842)	
HELICARIONIDAE	*Euconulus dentatus* (Sterki, 1893)	*
	Euconulus trochulus (Reinhardt, 1883)	*
	Guppya sterkii (Dall, 1888)	*
ZONITIDAE	*Mesomphix cupreus* (Rafinesque, 1831)	
	Mesomphix inornatus (Say, 1821)	
	Mesomphix perlaevis (Pilsbry, 1900)	
	Mesomphix vulgatus H. B. Baker, 1933	*
	Hawaiia miniscula (A. Binney, 1840)	*
	Paravitrea multidentata (A. Binney, 1840)	
	Paravitrea petrophila (Bland, 1883)	*
	Glyphyalinia indentata (Say, 1823)	
	Glyphyalinia cryptomphala (Clapp, 1915)	
	Glyphyalinia cumberlandiana (Clapp, 1919)	*
	Glyphyalinia wheatleyi (Bland, 1883)	
GASTRODONTIDAE	*Gastrodonta interna* (Say, 1822)	
	Striatura meridionalis (Pilsbry & Feriss, 1906)	*
	Ventridens demissus (A. Binney, 1843)	
	Zonitoides arboreus (Say, 1816)	
	Zonitoides lateumbilicatus (Pilsbry, 1895)	*
POLYGYRIDAE	*Allogona profunda* (Say, 1821)	*
	Catinella avara (Say, 1824)	*
	Euchemotrema fraternum (Say, 1824)	
	Inflectarius rugeli (Shuttleworth, 1852)	
	Appalachina sayana (Pilsbry, 1906)	
	Mesodon thyroidus (Say, 1816)	
	Mesodon zaletus (A. Binney, 1837)	
	Neohelix albolabris (Say, 1816)	
	Stenotrema angellum Hubricht, 1958	
	Stenotrema edvardsi (Bland, 1856)	
	Stenotrema hirsutum (Say, 1817)	*
	Stenotrema stenotrema (Pfieffer, 1819)	
	Triodopsis anteridon Pilsbry, 1940	*
	Triodopsis vulgata Pilsbry, 1940	*
	Xolotrema denotatum (Ferussac, 1821)	

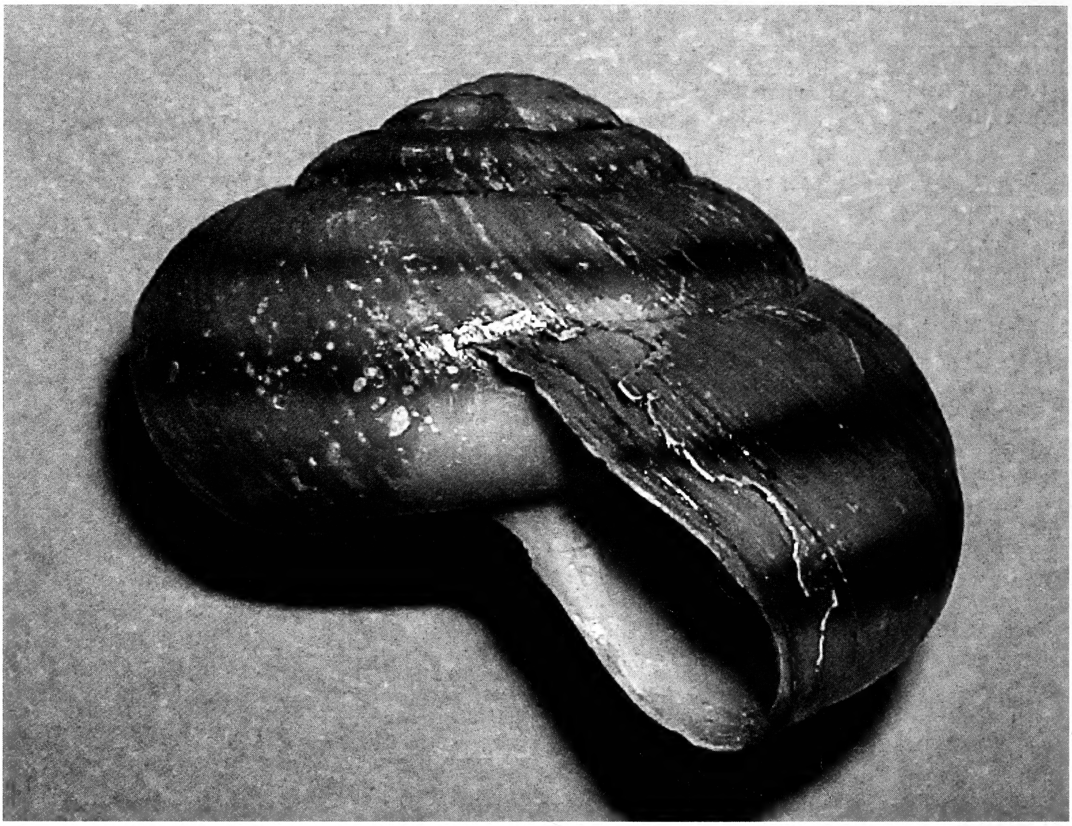

Figure 3. *Anguispira kochi*, a common land snail at Furnace Mountain, is currently on the unpublished watch list of the Kentucky State Nature Preserve Commission. Extant populations of this species are rare in Kentucky. Reasons for their decline are unknown.

(Pilsbry) (Figure 6), were not found despite intense searching. *P. appressa* is a common snail of limestone and sandstone cliff-lines, and, although the Furnace Mountain site had a fairly extensive limestone cliff-line, it was missing from this feature. The anomaly is interesting given that *P. appressa* is a common resident in similar habitats less than 1 km away. *T. complanata* also is a species common to calcareous regions of Central Kentucky (pers. obs.) and usually occurs in numbers along limestone rock outcrops. It is frequent elsewhere along the CKE of Furnace Mountain.

Two species, *Pomatiopsis lapidaria* (Say) and *Catinella avara* (Say) often referred to as amphibious or even aquatic (Hubricht, 1985), occupied moist leaf litter around the edges of the ephemeral pool. Small depressions that hold seasonal rain water in the limestone cap rock are relatively common occurrences on the CKE of eastern Kentucky. These seasonal wetlands often contain interesting assemblages of organisms, e.g. fairy shrimp, pea clams, and a variety of vertebrates and invertebrate larva.

Twenty-five micro-snails (<5 mm wide) were collected from leaf litter samples, representing 43% of the Furnace Mountain fauna. Micro-snails generally make up at least 25% of Kentucky collections but can be as much as 50% in rare cases (pers. obs.). Small snails are largely under represented in surveys. This was well illustrated in Hubricht's (1985) distribution records for eastern land snails that showed many county gaps for diminutive species. Adult shell sizes ranged from 1.5 mm, i.e. *Punctum minutissimum* (I. Lea) to 36 mm, *Neohelix albolabris* (Say).

After an evaluation of the literature on snail inventories across the world, Tattersfield (1996) concluded that sites of moderate sample size (approx. <10 ha) with at least 24

Figure 4. A familiar species to the Bluegrass, *Mesomphix vulgatus* is a land snail largely associated with Lexington limestone of low hills and ravines.

land snail species are of global conservation importance. Furnace Mountain is evidently among the richest global land snail communities having four more species than the 4.2 ha study site in New Zealand's Waipipi Scenic Reserve, a temperate rain forest containing 56 shelled species and one slug, the world's richest reported land snail assemblage (Solem

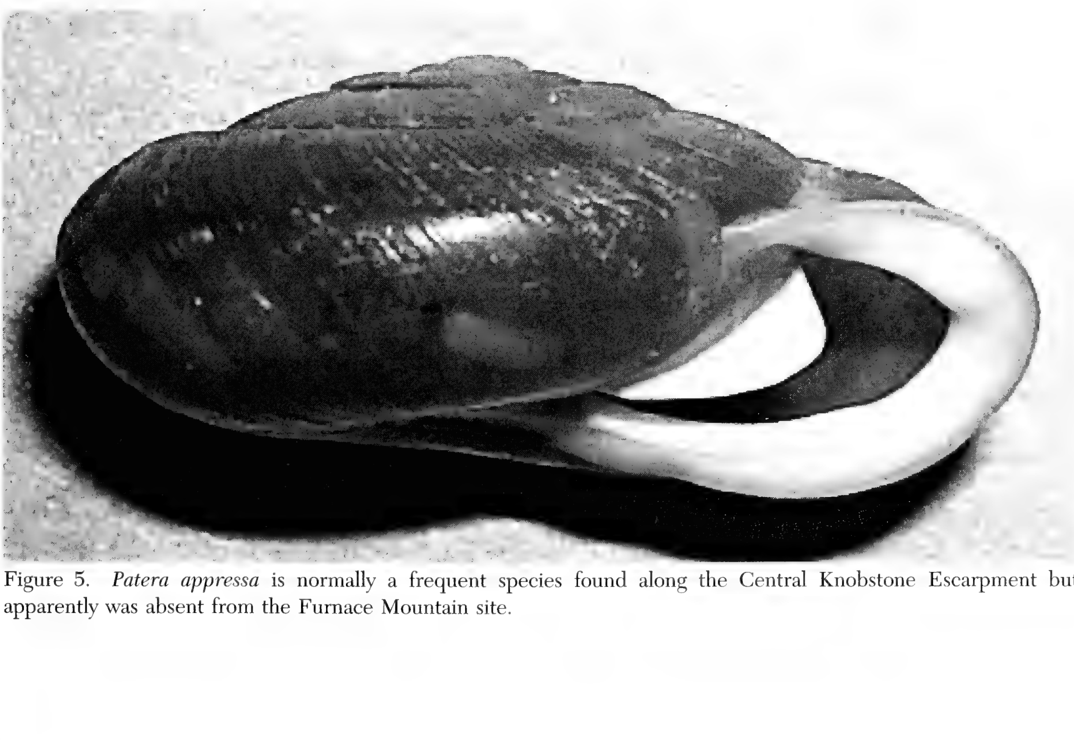

Figure 5. *Patera appressa* is normally a frequent species found along the Central Knobstone Escarpment but apparently was absent from the Furnace Mountain site.

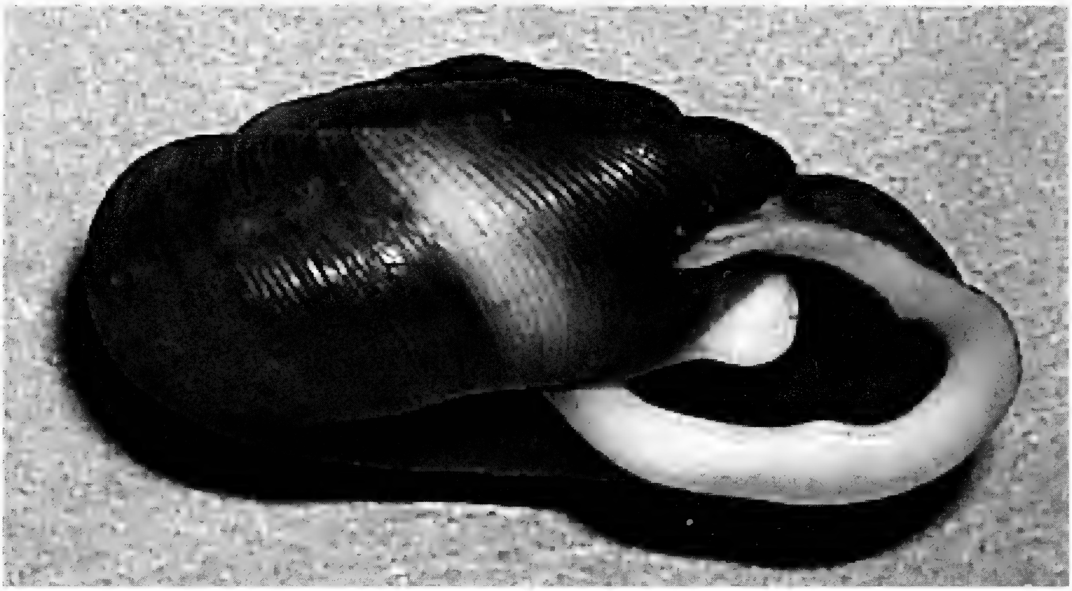

Figure 6. *Triodopsis complanata* is a common species to calcareous regions of Central Kentucky as well as the Central Knobstone Escarpment but was not documented at Furnace Mountain during this study. Its absence along with *Patera appressa* was one of the more interesting aspects of the study since both species can be found less than a kilometer in any direction.

et al. 1981). The Central Knobstone Escarpment section of Furnace Mountain holds diverse molluscan refugia for Kentucky and is globally significant.

DISCUSSION

Furnace Mountain, with a land snail fauna of 61 species, harbored 35% of the approximately 170 species that have been reported from Kentucky (Hubricht 1985; Branson 1973) and contained nearly all the regionally available fauna. When compared with land snail surveys across North America and globally, Furnace Mountain plainly exceeded a number of these studies. Research, however, that has focused on sample plots smaller than 2 ha turned out to be somewhat problematic when comparing data. Variations in methodologies and geographic size of inventoried areas complicate straightforward comparisons of species richness between studies. There also remains a great deal of uncertainly to what, in fact, characterizes a site. Nevertheless, a review of land snail research across the world is presented here.

Hubricht (1956) reported on land snails of the Shenandoah National Park, located in the Blue Ridge Mountains of northern Virginia. Hubricht collected 33 species of land snails from a variety of habitats ranging in elevations from 180 m to over 1220 m. This study area included a much larger sample size than that of the study area on Furnace Mountain.

Hubricht (1968) reported on the land snails of the Mammoth Cave National Park, Kentucky. This study documented 81 species from a variety of habitats including river bluffs, flood plains, ravines, sinks, and caves. Geologically, the study area was comprised of sandstone ridge summits, while the slopes of the ravines and the river bluffs were limestone. The study area collected was many times larger (samples were taken throughout the park), and the habitats were more varied than that of Furnace Mountain.

Branson and Batch (1968) surveyed land snails of Pine and Big Black Mountain, Kentucky forest habitats. Samples were collected from four sites and included 1) base of Pine Mountain, Pine Mountain State Park, Bell County, Ky.; 2) Big Black Mountain near Louellan, Harlan County, Kentucky; 3) top of Pine Mountain, 700–730 m Pine Mountain State Park, Bell County, Kentucky; and 4) Pine Mountain State Park Lodge, 457 m Bell County, Kentucky. Represented in their

collection were six families, 18 genera, and 47 species. This sample area included collections from two counties and four different sites at various elevations from mountain valleys to mountain summits.

Branson and Batch (1970) studied lands snails in the Mill Creek drainage located in Powell and Wolf Counties in Kentucky. Their study included 14 stations, stretching approximately 4 miles along forest and floodplain habitats of Mill Creek in the Middle Fork of the Red River. This site was located 6 air miles east of the Furnace Mountain study. Branson and Batch collected 1703 specimens. Represented in their collection were eight families, 21 genera, and 47 species of land snails. Most of what Branson and Batch collected corresponded with the Furnace Mountain fauna; however, there were some interesting differences. For example, they collected five *Paravitrea* species from the intersections of moist, deeply placed talus near the point where the slopes leveled out into the floor of the valley. The Furnace Mountain Site produced only two species of *Paravitrea* in spite of intense searching for this genus. The habitat at Mill Creek where Branson and Batch collected *Paravitrea* species did not occur at the Furnace Mountain site. Another faunal difference between Furnace Mountain and Mill Creek was in the Pupillidae. Furnace Mountain produced six species of Pupillidae whereas the Mill Creek site yielded only one. On Furnace Mountain, all Pupillidae species were found either entirely or largely on the extensive limestone outcrops that were so predominant. In contrast, the limestone outcrops at Mill Creek were small and broken into non-continuous features.

Petranka (1982) sampled land snails from 36 stations on Black Mountain, chosen to represent a variety of elevations and aspects. Twelve stations were established within each of three elevation zones: low 610–820 m; medium from 820–1035 m; and high from 1035–1250 m. A total of 12,464 individuals were collected representing 14 families, 26 genera, and at least 56 species. The collections included six new records for Kentucky, 21 new records for Harlan County, Kentucky, and at least one previously undescribed species. Indeed this was reported as one of the richest collected areas of the Eastern United States. Petranka's study encompassed approximately 776 ha and a much larger range of habitats than the Furnace Mountain site.

In 1998, a limited study of approximately 2 ha by Caldwell (unpublished data) in the Great Smoky Mountains National Park in the White Oak Sinks area documented 10 families, 12 genera, and 23 species of land snails. The area included several caves and limited limestone outcrops. In 2006, during the Karst Quest Inventory in conjunction with the All Taxa Biota Inventory (ATBI), 27 additional species were added to the White Oak Sinks snail list, bringing the total closer to 50. Caldwell's study area was similar in size to the Furnace Mountain site.

A 1999 land snail survey by Hotopp (unpublished data) from Cornwell Cave in West Virginia reported 35 species. The cave was deep in the Cheat River Gorge in Preston County. Hotopp collected snails at the main entrance of Cornwell Cave and at adjacent limestone outcrops and surrounding forest. The complete area searched was approximately 2 ha, the same size as the Furnace Mountain Site.

Sixty-three species of land snails were reported from a 25 ha block of calcareous woodland in southern Britain (Cameron et al. 2006). This represented nearly half the known terrestrial fauna of the country and may have held the richest forest fauna in Britain. At least eight species from the study, however, were reported as non-native inhabitants of British forests.

In Cameroon, Africa, 97 species were documented in a one square-kilometer patch of rainforest (deWinter and Gittenberger 1998). In the Great Lakes region of North America, 34 species were reported from a 0.10 ha plot (Nekola 1999). Forty land snails were reported from a single site in the Greater Antilles (Solem 1984), 24 species from a 0.01 ha area in the Italian Alps (Bishop 1980), 26 species from a 0.09 ha site in British Columbia coniferous forests (Cameron 1986), 27 species from a 9.1 ha island in Sweden (Nilsson et al. 1988), and at least 42 species from a 2 ha tropical rain forest in the Bladen Nature Reserve of southern Belize (Dourson, unpublished data).

A number of studies have focused on smaller sample sizes, sometimes documenting

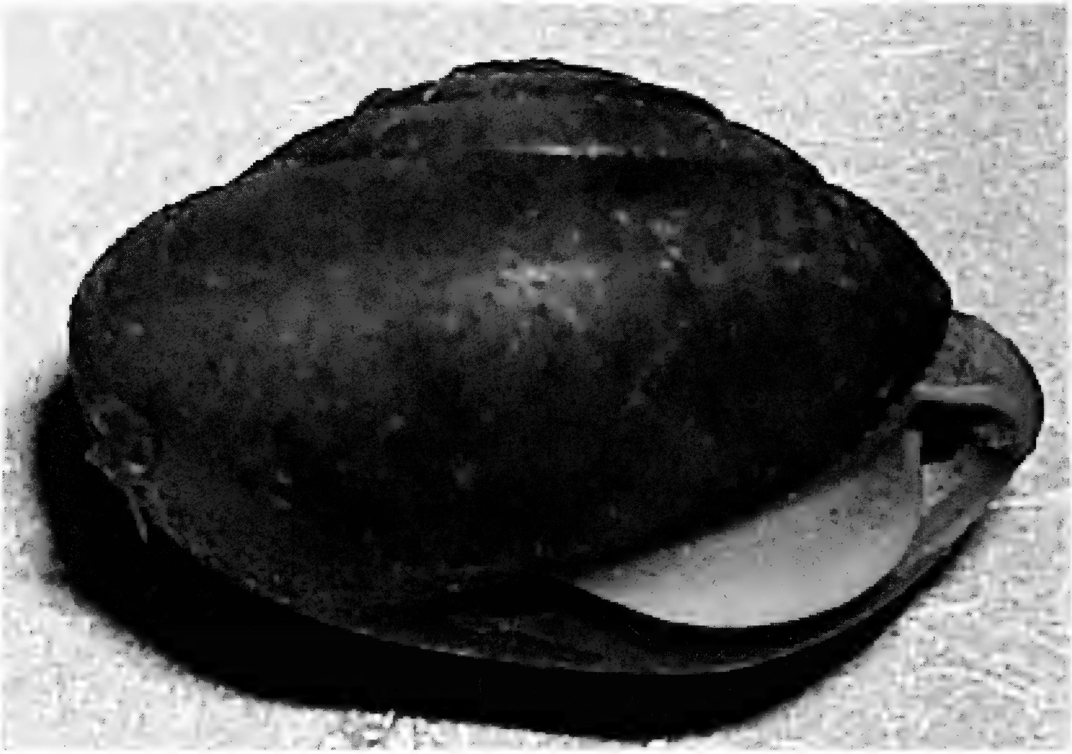

Figure 7. The physiographic diversity of the region has provided land snails like *Stenotrema angellum*, normally associated with the Bluegrass region, the opportunity to expand their distribution in Kentucky.

unusually high numbers of sympatric species co-existing in remarkably small places. Schmid (1966) reported 35 species including slugs on the Spitzberg near Tubingen from a single 1 m^2 quadrant. Cameron et al. (2006) reported that the difference between the numbers of snail species in their individual 400 m^2 plots and in clusters of such sites within a few kilometers of each other were slight. When the richest single sites were compared, the differences of snail faunas were even less. This suggested that relatively small areas could hold most of the locally occurring snail faunas. For example, in one of the Cameron et al. (2006) 25 ha study blocks, eight 400 m^2 plots were sampled from calcareous woodlands. The richest plot harbored 36 native snails, including slugs. Only eight additional species were added from the remaining seven sample plots for a total of 44 native snails occurring in the 25 ha block.

Caldwell's (unpublished) study in the Great Smoky Mountains and Hotopp's (unpublished) study at Cornwell Cave were the most similar to the Furnace Mountain site in terms of size, limestone habitat, and elevation. Although Mammoth Cave, Cameroon, and Hiawassee sites reported higher numbers of species collected, they each represented a substantially greater area sampled.

Several factors contributed to the unusually high number of snails occurring on Furnace Mountain. Most significant were likely to be the far-reaching limestone cliff-lines and the associated carbonate rich soils. Nekola (1999) reported that the richest snail sites in the Great Lakes Region were carbonate cliffs. Snail diversity and shell abundance largely correlated with available soil and leaf tissue calcium (Burch 1955; Cameron 1986; Wareborn 1992; Hotopp 2002; Nation 2007). Abcissed leaves containing substantial foliar calcium content include yellow birch *Betula allegheniensis* Britton and the flowering dogwood (Rickleps and Matthew 1982; Nation 2007). Land snails obtain calcium by ingesting soil particles, rasping calcium-rich limestone, and digesting decaying leaf matter (Wareborn 1979, 1992; Fournie and Chetail 1984; Burch and Pearce 1990; Nation 2007). Calcium

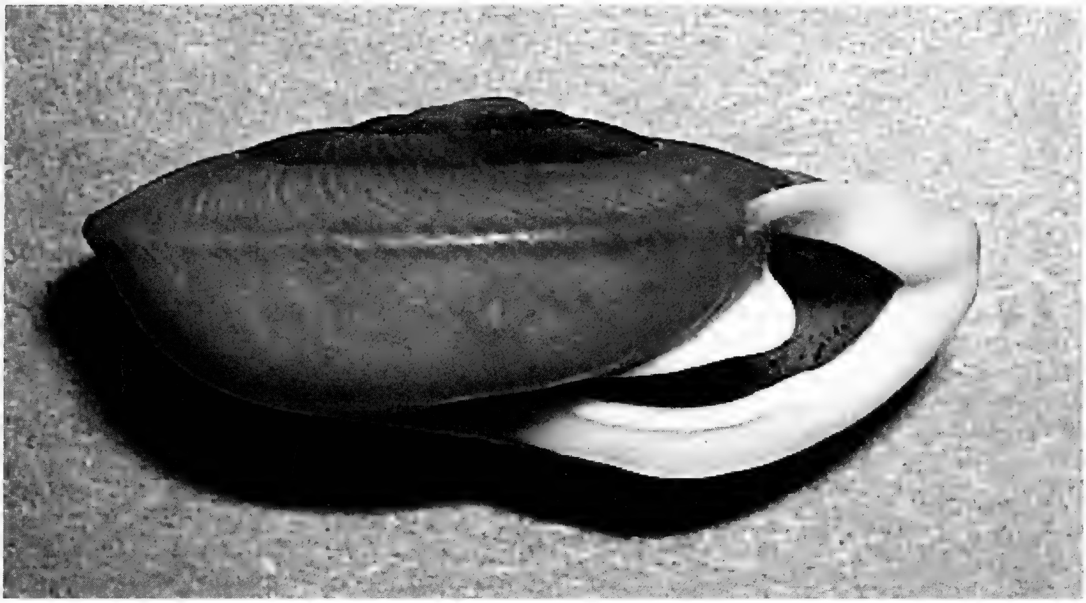

Figure 8. The *Patera* species shown in Figure 8 represents either a unique ecological form of *Patera appressa* or is an entirely new species to science. The species, distinguished by its carinate periphery, is found 24 km to the south of Furnace Mountain in the Central Knobstone Escarpment.

carbonate is required by land snails for reproduction and regulation of bodily functions but most importantly in shell-building (Burch 1962; Fournie and Chetail 1984; and Hickman et al. 2003). Given that the study area contained calcareous-based soils, it was not surprising that many of the species represented were calciphile snails, e.g. *Pomatiopsis lapidaria*, *Vallonia perspectiva*, *Gastrocopta corticaria* (Say), and *Pupoides albolabris* (C. B. Adams) (Hubricht 1985).

Species richness on Furnace Mountain is thought to have been largely driven by the calcium rich soils, but other important influences likely have played a role. The Central Knobstone Escarpment forms a large geophysical landscape edge where the Cumberland Plateau, the Knobs, and the Outer Bluegrass regions of Kentucky converge. These geologic landscapes by themselves contain their own distinct snail affiliations. The physiographic diversity of the region has provided snails a window of opportunity to expand their distributions and habitats in Kentucky. Snails that are not usually found together were found co-existing there. Two species, *Stenotrema angellum* Hubricht (Figure 7) and *Mesomphix vulgatus,* that typically inhabit low Lexington limestone hills in the Bluegrass, were found living among Newman limestones high on the edge of the Cumberland Plateau.

The extensive and continuous limestone cliff-lines or walls that dominate this region may have driven land snail distribution patterns by channeling their movements and by concentrating species richness along these rock outcrops. Clearly the greatest diversity and numbers of snails occurred within 50 m of the cliff-line features and species richness and numbers dropped dramatically beyond this point. Kalisz and Powell (2003) reported the effects of calcareous road dust on land snails and millipedes (Diplopoda) in acidic forest soils in the Daniel Boone National Forest of Kentucky. Soil pH decreased from 7.0 to 4.3 with increasing distance from the road. Dry snail mass was approximately 10× higher at the roadside than 50 m away.

Furnace Mountain also maintained a multiplicity of temperature and moisture-related habitats from cool north-facing slopes to hot relatively dry outcrops of limestone that provided conditions suitable for a range of land snail preferences. For example, *Carychium* species were largely found living between the layers of cool, moist leaf litter, a common habitat below the cliff-line. In

contrast, *Strobilops labyrinthica* (Say) preferred the exceptionally dry, thin detritus found around red cedar trees located on the more exposed cliff-line features. Other examples of species partitioning as a function of leaf moisture content or exposed rock were commonly witnessed. The greatest numbers of Valloniidae and Pupillidae species were found on the dry, glade-like habitats of the limestone cliffs. Below the main cliff wall where leaf litter and detritus deposits were deep, Punctidae, Philomycidae, Zonitidae, and Polygyridae were in the majority.

The exceptional land snail diversity of Furnace Mountain was not likely exclusive to the region but indicative of the Central Knobstone Escarpment (CKE) of Kentucky as a whole. This, however, remains speculative awaiting further investigation. The north-facing hillside of Furnace Mountain is reminiscent of countless other hillsides along the escarpment. Other portions of the CKE have been known to contain high species richness in addition to harboring unique snails (Dourson unpublished data). An undescribed land snail in the genus *Patera*, (Polygyridae) (Figure 8) was found 24 km to the south along the CKE in Estill County. It appeared to be endemic to this region, known only from a few ridges within several square kilometers. The species is distinctive as it is one of but a few land snails in North America that possesses a carinate periphery.

The Furnace Mountain section of the Central Knobstone Escarpment is recommended for inclusion into Kentucky's rare molluscan biodiversity eco-regions.

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Annotated List of the Leaf Beetles (Coleoptera: Chrysomelidae) of Kentucky: Subfamily Cassidinae

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ABSTRACT

An examination of leaf beetle specimens (Coleoptera: Chrysomelidae) in the five largest beetle collections in Kentucky and from recent inventory work in state nature preserves revealed 31 species of the subfamily Cassidinae present in Kentucky, 13 of which are previously unreported for the state. Distribution maps and label data are presented for the 31 Kentucky species of the subfamily Cassidinae including spatial (state and Kentucky county records), temporal (years and months of collection in Kentucky), and plant association information. The following species are reported from Kentucky for the first time: *Anisostena ariadne* (Newman), *Anisostena nigrita* (Olivier), *Microrhopala excavata excavata* (Olivier), *Microrhopala rileyi* S. Clark, *Odontota horni* J. Smith, *Sumitrosis ancoroides* (Schaeffer), *Physonota unipunctata* (Say), *Cassida rubignosa* Müller, *Gratiana pallidula* (Boheman), *Erepsocassis rubella* (Boheman), *Jonthonota nigripes* (Olivier), *Opacinota bisignata* (Boheman), and *Strongylocassis atripes* (LeConte).

KEY WORDS: Kentucky, leaf beetles, Chrysomelidae, biodiversity, new state records

INTRODUCTION

To better understand, appreciate, document, and, we hope, conserve the fauna of Kentucky, an inventory of the leaf beetles (Coleoptera: Chrysomelidae) was initiated in 2004. Chrysomelids, which are almost exclusively plant feeders, are one of the largest insect families (~40,000 species worldwide, Jolivet and Verma 2002). The evolution of leaf beetles is believed to be linked with the evolution of flowering plants (angiosperms) (Farrell 1998). Many leaf beetles are plant-genus or plant-family specific in their food choice, and many have become agricultural pests, while others are remnant-dependent species found only on rare occasions (Panzer et. al. 1995). The diversity of chrysomelids in an area or region should be related to the diversity of its plant community. Leaf beetles, many of which are easily collected, can be used as indicator species for biodiversity studies. This paper is the first of a series that will present a synopsis of the historical collection data from the major Coleoptera collections in Kentucky and augment these data with new information gained from recent and ongoing intensive monitoring at state preserves. Following in the tradition of the Society of Kentucky Lepidopterists (Gibson and Covell 2006), the inventory will be periodically updated as new records become available.

The subfamily Cassidinae now includes two formerly recognized subfamilies: Hispinae, the leaf-mining 'hispines', and Cassidinae, the tortoise beetles. Staines (2006) recently published a species level review of the leaf mining tribes, and Riley (1986) provided a genus level review of the tribe Cassidini that includes most of the tortoise beetle genera found in the United States. The purpose of the present study is to present data from the labels of cassidine leaf beetle specimens known from Kentucky, specifically spatial information (state and county records),

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temporal information (years and months of collection in Kentucky), plant associations, and collection methods. Comments are provided for most species and include information on abundance and known and probable host plants.

MATERIALS AND METHODS

To establish a historical perspective, leaf beetle specimens from the major insect collections in Kentucky (and from collections located in other states, but known to contain Kentucky specimens) were examined, reidentified, and their label data recorded. The following collections were studied: the University of Kentucky Insect Collection (UKIC) that contains the Charles V. Covell, Jr. Collection (emeritus professor of the University of Louisville); the private collection of Robert J. Barney (RJBC) that comprises two time periods of collecting in Kentucky, 1976–1984 and 2004–2006; the private collection of Charles Wright, the Kentucky Beetles Project Collection (CWC) that was established in 1991 in an effort to document Coleoptera within the state; the Western Kentucky University Collection (WKUC); the Brigham Young University Collection (BYUC); the Colorado State University Collection (CSUC); and the Kentucky State University Insect Collection (KSUC) that houses the specimens generated by the Kentucky Leaf Beetle Biodiversity Project. In this project we are currently conducting extensive collecting in many grassland-dominated barrens and rock outcrop (glade) communities that are known for possessing uncommon plants and plant associations (Jones 2005). These sites are primarily in state nature preserves that have never been surveyed for plant-feeding beetles. Most specimens were collected by the senior author within five state nature preserves in 2004–2006: Crooked Creek Barrens (Lewis County) and Blue Licks Battlefield (Robertson County) in northeastern Kentucky, Eastview Barrens (Hardin County) and Thompson Creek Glades (LaRue County) in central Kentucky, and Raymond Athey Barrens (Logan County) in western Kentucky.

For each cassidine species here documented for Kentucky, the following data are presented: state-level distribution in the United States (from Riley et al. 2003), Kentucky county records, abundance by year and month in Kentucky, and specimens per collection. Other pertinent information from specimen labels, such as the method of collection and plant association information, is presented in the "Comments" section for each species. This information provides the opportunity to determine abundance, seasonality, and distribution from a historical perspective. Relative abundance and rare species status are subjective judgments based on frequency of occurrence and intensity of collecting efforts and follow Jones (2005). Plant collection records taken from specimen labels are notoriously inaccurate in many cases and may not reflect true host plants (Clark et al. 2004).

RESULTS

According to the "Catalog of Leaf Beetles of America North of Mexico" (Riley et al. 2003), there are 47 species of Cassidinae recorded in at least one of the seven states contiguous to Kentucky, thus establishing this number as a "ballpark" estimate for the state. However, in that work only 19 species were listed from Kentucky. An examination of 1,203 cassidine leaf beetle specimens from the major collections in the state (and from collections located outside the state, but known to contain Kentucky specimens) revealed 31 species, including 18 of the 19 recorded in Riley et al. (2003) plus 13 new state records (Table 1).

The state collection at the University of Kentucky (UKIC) contains a total of 458 cassidine leaf beetles representing 19 species, including two of the new state records reported herein. This collection contains the oldest in-state specimen records for Kentucky leaf beetles, with collection dates as early as 1889. The CWC collection has 65 specimens representing 14 species including one of the new state records reported herein. The collection at WKUC has 12 specimens of three species. Recent collecting in state nature preserves (the KSUC collection) has produced 482 specimens of 22 species including four of the new state records reported herein. The RJBC collection contains 130 specimens of 20 species from Kentucky, including five of the new state records reported herein. While examining the RJBC Cassidinae, four addi-

Table 1. List of cassidine leaf beetles (Coleoptera: Chrysomelidae) recorded from Kentucky with indications of number of specimens examined, number of Kentucky county records, years of collection, and new state records indicated.

Tribe Cephaloleiini	
Stenispa metallica (F.)	27 specimens: 8 counties, 1981–2006
Tribe Chalepini	
Anisostena ariadne (Newman)	39 specimens: 1 county, 2005–2006 (new state record)
Anisostena nigrita (Olivier)	77 specimens: 4 counties, 1983–2006 (new state record)
Baliosus nervosus (Panzer)	6 specimens: 5 counties, 1892–1995
Chalepus bicolor (Olivier)	17 specimens: 9 counties, 1968–2006
Glyphuroplata pluto (Newman)	4 specimens: 4 counties, 1942–1987
Microrhopala excavata excavata (Olivier)	6 specimens: 3 counties, 1983–2006 (new state record)
Microrhopala hectate (Newman)	1 specimen: 1 county, 1975
Microrhopala rileyi S. Clark	22 specimens: 1 county, 2005–2006 (new state record)
Microrhopala vittata (F.)	31 specimens: 9 counties, 1938–2006
Microrhopala xerene (Newman)	6 specimens: 3 counties, 1994–2005
Octotoma plicatula (F.)	34 specimens: 5 counties, 1909–2006
Odontota dorsalis Thunberg	263 specimens: 31 counties, 1889–2006
Odontota horni J. Smith	40 specimens: 5 counties, 1983–2006 (new state record)
Odontota scapularis (Olivier)	17 specimens: 10 counties, 1947–2005
Sumitrosis ancoroides (Schaeffer)	3 specimens: 2 counties, 2005–2006 (new state record)
Sumitrosis inaequalis (Weber)	69 specimens: 19 counties, 1951–2006
Sumitrosis rosea (Weber)	13 specimens: 3 counties, 1981–2005
Tribe Mesomphaliini	
Chelymorpha cassidea (F.)	43 specimens: 11 counties, 1890–2006
Tribe Ischyrosonychini	
Physonota unipunctata (Say)	1 specimen: 1 county, 1996 (new state record)
Tribe Cassidini	
Agroiconota bivittata (Say)	90 specimens: 16 counties, 1891–2006
Cassida rubiginosa Müller	4 specimens: 3 counties, 1994–2004 (new state record)
Deloyala guttata (Olivier)	120 specimens: 25 counties, 1891–2006
Gratiana pallidula (Boheman)	2 specimens: 2 counties, 1895–1993 (new state record)
Plagiometriona clavata clavata (F.)	9 specimens: 4 counties, 1892–2006
Charidotella purpurata (Boheman)	23 specimens: 8 counties, 1971–2006
Charidotella sexpunctata bicolor (F.)	145 specimens: 28 counties, 1891–2006
Erepsocassis rubella (Boheman)	16 specimens: 1 county, 2005–2006 (new state record)
Jonthonota nigripes (Olivier)	5 specimens: 3 counties, 1966–2006 (new state record)
Opacinota bisignata (Boheman)	29 specimens: 4 counties, 1912–2006 (new state record)
Strongylocassis atripes (LeConte)	42 specimens: 4 counties, 1985–2006 (new state record)

tional new state records for states other than Kentucky were found. These have been added to the distribution maps and are cited in the "Comments" section for those species. An examination of the BYUC revealed 54 specimens in 14 species. The authors were aware also of two leaf beetles in CSUC collected in Kentucky, one of which is a state record for Kentucky reported herein.

Stenispa metallica (F.) (Figure 1A)

Kentucky Counties: Fayette, Grayson, Hardin, Jefferson, LaRue, Lewis, Lincoln, Logan

Years: 1981 (4), 1983 (2), 2004 (1), 2005 (15), 2006 (5)

Months: April (3), May (17), June (5), July (2)

Abundance: 27 specimens: 21-KSUC, 5-RJBC, 1-UKIC

Comments: This species occurs in wetlands, where it may be associated with sedges. A new state record was found for Illinois in the RJBC (7 specimens from Grundy, Pulaski and Will Counties) (Figure 1A).

Baliosus nervosus (Panzer) (Figure 1B)

Kentucky Counties: Breathitt, Fayette, Jefferson, Rowan, Whitley

Years: 1892 (1), 1956 (1), 1975 (1), 1984 (1), 1994 (1), 1995 (1)

Months: April (1), May (2), June (2), July (1)

Abundance: 6 specimens: 2-BYUC, 1-CWC, 1-RJBC, 2-UKIC

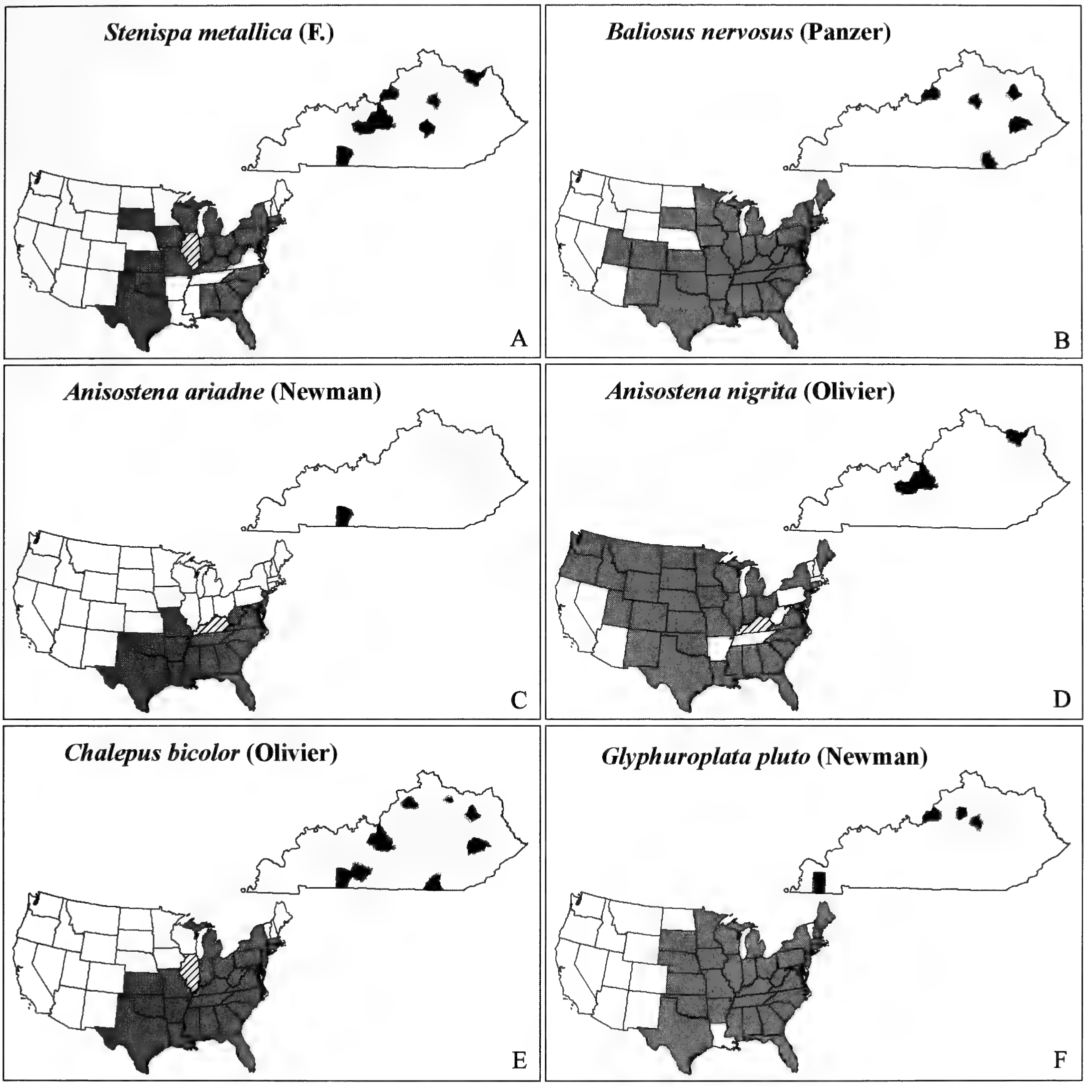

Figure 1. The known distribution of Cassidinae leaf beetles (Coleoptera: Chrysomelidae) is illustrated in grey shading in Kentucky counties and the United States. New state records reported herein are shown in cross-hatch.

Comments: Although commonly called the basswood leafminer, these beetles can be also found on many other plants.

Anisostena ariadne (Newman) (Figure 1C)

Kentucky Counties: Logan

Years: 2005 (12), 2006 (27)

Months: May (14), June (12), July (11), August (2)

Abundance: 39 specimens: 39-KSUC (new state record) (Figure 1C)

Comments: All specimens examined were from the Raymond Athey Barrens State Nature Preserve. Ford and Cavey (1982) reported *Panicum virgatum* L. (switch grass) as a host plant.

Anisostena nigrita (Olivier) (Figure 1D)

Kentucky Counties: Grayson, Hardin, LaRue, Lewis

Years: 1983 (3), 2004 (36), 2005 (27), 2006 (11)

Months: April (2), May (17), June (29), July (22), August (3), September (4)

Abundance: 77 specimens: 74-KSUC, 3-RJBC (new state record) (Figure 1D)

Comments: All specimens examined were recently collected in prairie-type habitats in

nature preserves. This species mines leaves of *Schizachyrium scoparium* (Michx.) Nash (Clark 2000).

Chalepus bicolor (Olivier) (Figure 1E)

Kentucky Counties: Breathitt, Hardin, Henry, LaRue, Logan, McCreary, Robertson, Rowan, Warren
Years: 1968 (1), 1972 (1), 1983 (1), 1990 (1), 2004 (1), 2005 (8), 2006 (4)
Months: May (8), June (5), July (4)
Abundance: 17 specimens: 1-BYUC, 13-KSUC, 1-RJBC, 1-UKIC, 1-WKUC
Comments: One label reported collection by Malaise trap. This species is associated with *Dichanthelium* (Poaceae) (Clark et al. 2004). A new state record was found for Illinois in the RJBC (4 specimens from Jackson, Mason and Schuyler Counties) (Figure 1E). Staines (1995) reported *Chalepus bacchus* (Newman) from Henderson County, Kentucky. We have some reservations about the status of *C. bacchus* as presently defined because the diagnostic character proposed to separate this species from *C. bicolor* does not hold true upon examination of many specimens. All specimens of the genus *Chalepus* that we examined from Kentucky appear to represent a single species, *C. bicolor.*

Glyphuroplata pluto (Newman) (Figure 1F)

Kentucky Counties: Fayette, Franklin, Graves, Jefferson
Years: 1942 (1), 1971 (1), 1987 (1)
Months: May (2), July (1)
Abundance: 3 specimens: 1-RJBC, 2-UKIC
Comments: The Jefferson County record was reported by Riley (1985b) from Louisville. This species is associated with grasses.

Microrhopala excavata excavata (Olivier) (Figure 2A)

Kentucky Counties: Grayson, LaRue, Whitley
Years: 1983 (1), 2005 (3), 2006 (2)
Months: April (1), May (4), July (1)
Abundance: 6 specimens: 5-KSUC, 1-RJBC (new state record) (Figure 2A)
Comments: This subspecies was first found in Kentucky in a small railroad prairie near Leitchfield, Grayson County. This subspecies feeds on *Doellingeria umbellata* (Mill.) Nees (flat-topped white aster) and *Solidago* sp. (goldenrod) (Clark 1983).

Microrhopala hectate (Newman) (Figure 2B)

Kentucky Counties: Rowan
Years: 1975 (1)
Months: May (1)
Abundance: 1 specimen: 1-BYUC (Figure 2B)
Comments: Staines (2006) reported the larval host plant as unknown.

Microrhopala rileyi S. Clark (Figure 2C)

Kentucky Counties: Logan
Years: 2005 (5), 2006 (17)
Months: May (13), June (6), July (3)
Abundance: 22 specimens: 22-KSUC (new state record) (Figure 2C)
Comments: All specimens were collected at the Raymond Athey State Nature Preserve or Logan County State Nature Preserve. This species is reported to feed on *Helianthus* spp. (sunflower) (Clark 1983).

Microrhopala vittata (F.) (Figure 2D)

Kentucky Counties: Anderson, Boone, Hardin, Jefferson, LaRue, Lewis, Logan, Madison, Scott
Years: 1938 (1), 1971 (3), 1981 (3), 1992 (1), 1993 (4), 2004 (6), 2005 (5), 2006 (8)
Months: April (4), May (4), June (10), July (10), August (2), September (1)
Abundance: 31 specimens: 4-BYUC, 1-CWC, 19-KSUC, 2-RJBC, 5-UKIC
Comments: Species of *Solidago* (goldenrod) are the preferred hosts (Clark 1983).

Microrhopala xerene (Newman) (Figure 2E)

Kentucky Counties: Hardin, Jackson, LaRue
Years: 1994 (1), 2000 (1), 2004 (1), 2005 (3)
Months: April (1), May (3), June (1), July (1)
Abundance: 6 specimens: 1-BYUC, 1-CWC, 4-KSUC
Comments: Species of *Aster* are preferred to other hosts (Clark 1983).

Odontota dorsalis Thunberg (Figure 2F)

Kentucky Counties: Anderson, Boyd, Boyle, Bracken, Breckinridge, Bullitt, Estill, Fayette, Franklin, Grant, Grayson, Hardin, Jackson, Jefferson, Kenton, Knott, Knox, Lewis, Logan, Madison, Meade, Mercer, Oldham, Owen, Pendleton, Perry, Powell, Pulaski, Robertson, Shelby, Whitley

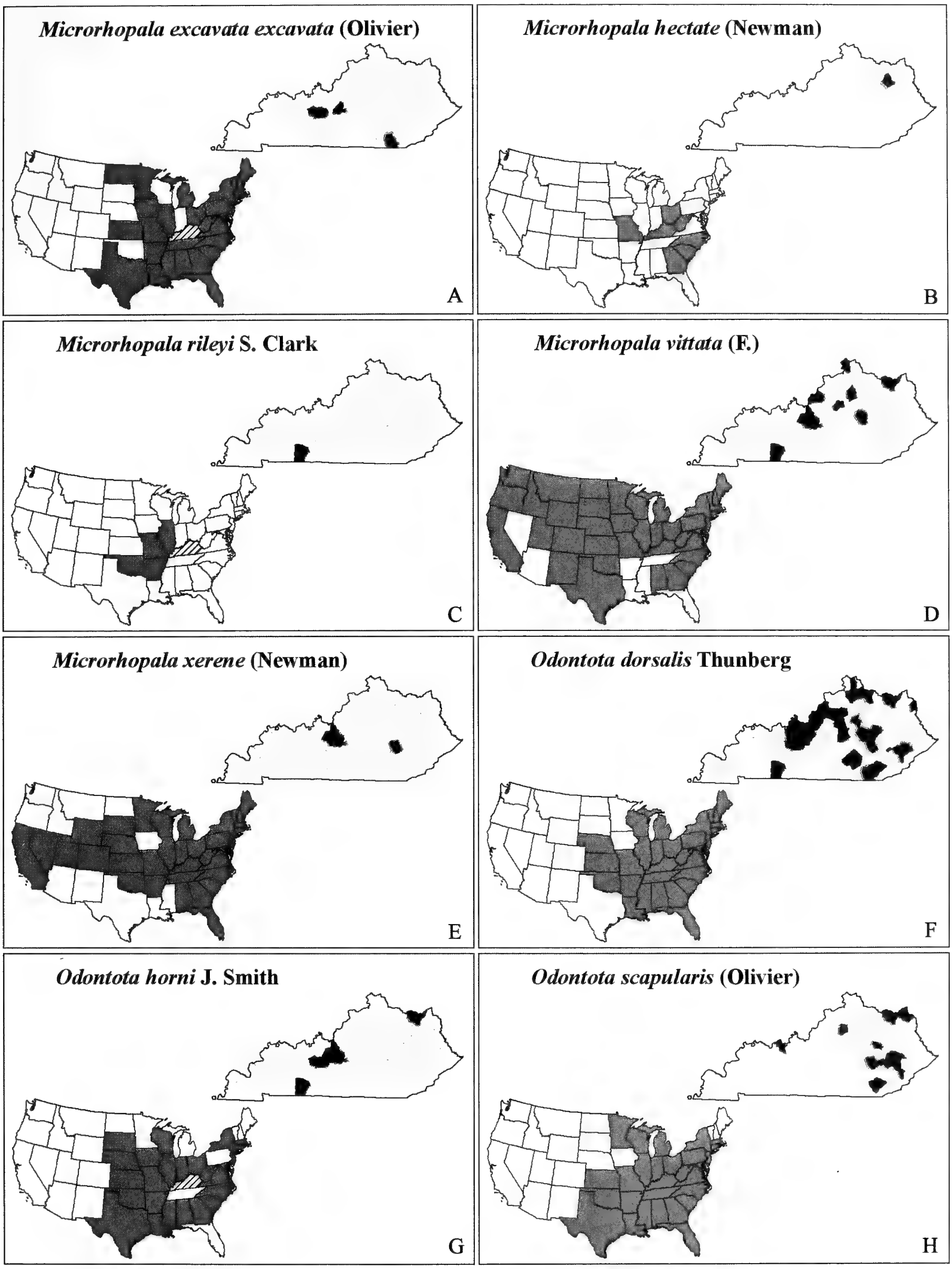

Figure 2. The known distribution of Cassidinae leaf beetles (Coleoptera: Chrysomelidae) is illustrated in grey shading in Kentucky counties and the United States. New state records reported herein are shown in cross-hatch.

Years: 1889 (10), 1891 (14), 1894 (6), 1895 (3), 1912 (1), 1916 (4), 1923 (12), 1938 (23), 1939 (12), 1943 (1), 1944 (1), 1946 (7), 1947 (23), 1948 (30), 1950 (1), 1959 (2), 1966 (2), 1967 (6), 1968 (2), 1970 (3), 1971 (6), 1972 (21), 1974 (8), 1975 (6), 1976 (5), 1979 (2), 1981 (14), 1984 (2), 1985 (1), 1988 (3), 1990 (3), 1992 (1), 1994 (2), 1995 (5), 1996 (1), 1997 (2), 1998 (7), 2001 (3), 2003 (3), 2004 (2), 2005 (1), 2006 (2)

Months: February (3), March (6), April (24), May (93), June (31), July (96), August (7), December (3)

Abundance: 263 specimens: 6-BYUC, 12-CWC, 5-KSUC, 20-RJBC, 220-UKIC

Comments: This species is very common and sometimes called the leaf-mining locust beetle. Butte (1968) reported four specimens from Kentucky in Harvard MCZ with no further data. Label data in UKIC reported associations with black locust, *Robinia pseudoacacia* L. and soybean, and collection by light trap and Malaise trap.

Odontota horni J. Smith (Figure 2G)

Kentucky Counties: Grayson, Hardin, LaRue, Lewis, Logan

Years: 1983 (1), 1985 (2), 2004 (8), 2005 (17), 2006 (12)

Months: May (16), June (14), July (9), August (1)

Abundance: 40 specimens: 37-KSUC, 3-RJBC (new state record) (Figure 2G)

Comments: This species was first found in Kentucky in a small railroad prairie near Leitchfield, Grayson County. This species is normally associated with Fabaceae (Clark et al. 2004).

Odontota scapularis (Olivier) (Figure 2H)

Kentucky Counties: Breathitt, Franklin, Greenup, Hancock, Jackson, Knox, Lewis, Owsley, Perry, Powell

Years: 1947 (3), 1984 (3), 1987 (1), 1992 (1), 1992 (1), 1994 (2), 1997 (2), 1998 (1), 2004 (2), 2005 (1)

Months: May (7), June (6), July (4)

Abundance: 17 specimens: 4-BYUC, 6-CWC, 4-RJBC, 3-UKIC

Comments: Clark et al. (2004) reported the normal host as *Apios americana* Medik., but many other associations have been cited.

Octotoma plicatula (F.) (Figure 3A)

Kentucky Counties: Caldwell, Fayette, Franklin, Fulton, Powell

Years: 1909 (4), 1910 (3), 1913 (1), 1947 (1), 1969 (10), 1971 (3), 1973 (1), 1975 (1), 2005 (6), 2006 (4)

Months: May (16), June (6), July (1), August (6), September (3), October (2)

Abundance: 34 specimens: 10-RJBC, 24-UKIC

Comments: Many labels reported *Campsis (Tecoma) radiacans* (L.) Seem. ex Bureau, trumpet creeper, as host.

Sumitrosis ancoroides (Schaeffer) (Figure 3B)

Kentucky Counties: Lincoln, Logan

Years: 2005 (1), 2006 (2)

Months: May (2), June (1)

Abundance: 3 specimens: 3-KSUC (new state record) (Figure 3B)

Comments: This species was first found in Kentucky at Bouteloua Barrens State Nature Preserve, Lincoln County. *Strophostyles* spp. are known as host plants (Clark et al. 2004).

Sumitrosis inaequalis (Weber) (Figure 3C)

Kentucky Counties: Boyd, Carter, Daviess, Estill, Franklin, Greenup, Hancock, Hardin, Jefferson, LaRue, Lee, Lewis, Logan, Martin, Meade, Menifee, Owsley, Rowan, Russell

Years: 1951 (1), 1952 (1), 1954 (3), 1981 (8), 1983 (11), 1984 (3), 1990 (4), 1992 (1), 1994 (5), 1995 (1), 1998 (2), 2002 (3), 2003 (3), 2004 (4), 2005 (6), 2006 (13)

Months: April (6), May (46), June (12), July (5)

Abundance: 69 specimens: 11-BYUC, 12-CWC, 19-KSUC, 22-RJBC, 5-UKIC

Comments: A UKIC specimen label reported an association with *Eupatorium fistulosum* Barratt (hollow-stemmed joe-pye weed).

Sumitrosis rosea (Weber) (Figure 3D)

Kentucky Counties: Jefferson, LaRue, Lewis

Years: 1981 (11), 1998 (1), 2005 (1)

Months: May (11), July (2)

Abundance: 13 specimens: 1-BYUC, 1-KSUC, 10-RJBC, 1-UKIC

Comments: This species is normally associated with Fabaceae (Clark et al. 2004).

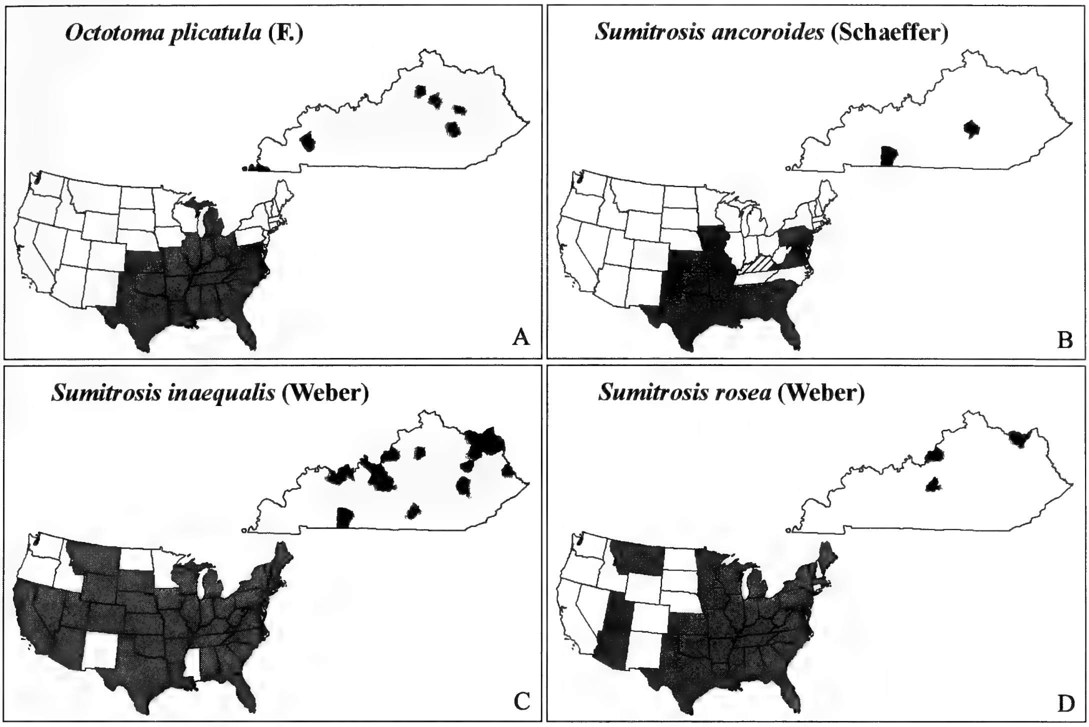

Figure 3. The known distribution of Cassidinae leaf beetles (Coleoptera: Chrysomelidae) is illustrated in grey shading in Kentucky counties and the United States. New state records reported herein are shown in cross-hatch.

Chelymorpha cassidea (F.) (Figure 4A)

Kentucky Counties: Boone, Fayette, Grant, Hardin, Henry, Jefferson, Lewis, Logan, Owen, Pendleton, Washington

Years: 1890 (1), 1941 (2), 1944 (1), 1948 (3), 1951 (1), 1952 (1), 1955 (1), 1966 (2), 1967 (1), 1971 (6), 1980 (1), 1981 (3), 1998 (1), 2003 (1), 2004 (1), 2005 (4), 2006 (13)

Months: April (1), May (7), June (23), July (9), August (1), September (2)

Abundance: 43 specimens: 1-BYUC, 2-CWC, 17-KSUC, 23-UKIC

Comments: The common name for this abundant species is the argus tortoise beetle.

Physonota unipunctata (Say) (Figure 4B)

Kentucky Counties: Clay

Years: 1996 (1)

Month: April (1)

Abundance: 1 specimen: 1-CWC (new state record) (Figure 4B)

Comments: This species is rare in Kentucky and has been cited as feeding on *Monarda* (Laminiaceae) (Clark et al. 2004).

Agroiconota bivittata (Say) (Figure 4C)

Kentucky Counties: Bath, Carroll, Fayette, Franklin, Grayson, Hardin, Harrison, Jefferson, LaRue, Lewis, Logan, Monroe, Nicholas, Owen, Washington, Wolfe

Years: 1891 (4), 1927 (1), 1938 (1), 1941 (3), 1947 (1), 1965 (1), 1971 (4), 1981 (3), 1983 (1), 1994 (1), 1995 (1), 1998 (3), 2003 (3), 2004 (9), 2005 (22), 2006 (32)

Months: March (1), May (18), June (47), July (21), August (2), September (1)

Abundance: 90 specimens: 4-BYUC, 6-CWC, 59-KSUC, 6-RJBC, 15-UKIC

Comments: The common name of this frequently collected species is the striped tortoise beetle. A UKIC specimen label reported occurrence on leaves of sweet potato.

Cassida rubiginosa Müller (Figure 4D)

Kentucky Counties: Hart, Martin, Rowan

Years: 1994 (1), 2003 (2), 2004 (1)

Months: April (1), May (1), June (2)

Abundance: 4 specimens: 1-CSUC, 3-CWC (new state record) (Figure 4D)

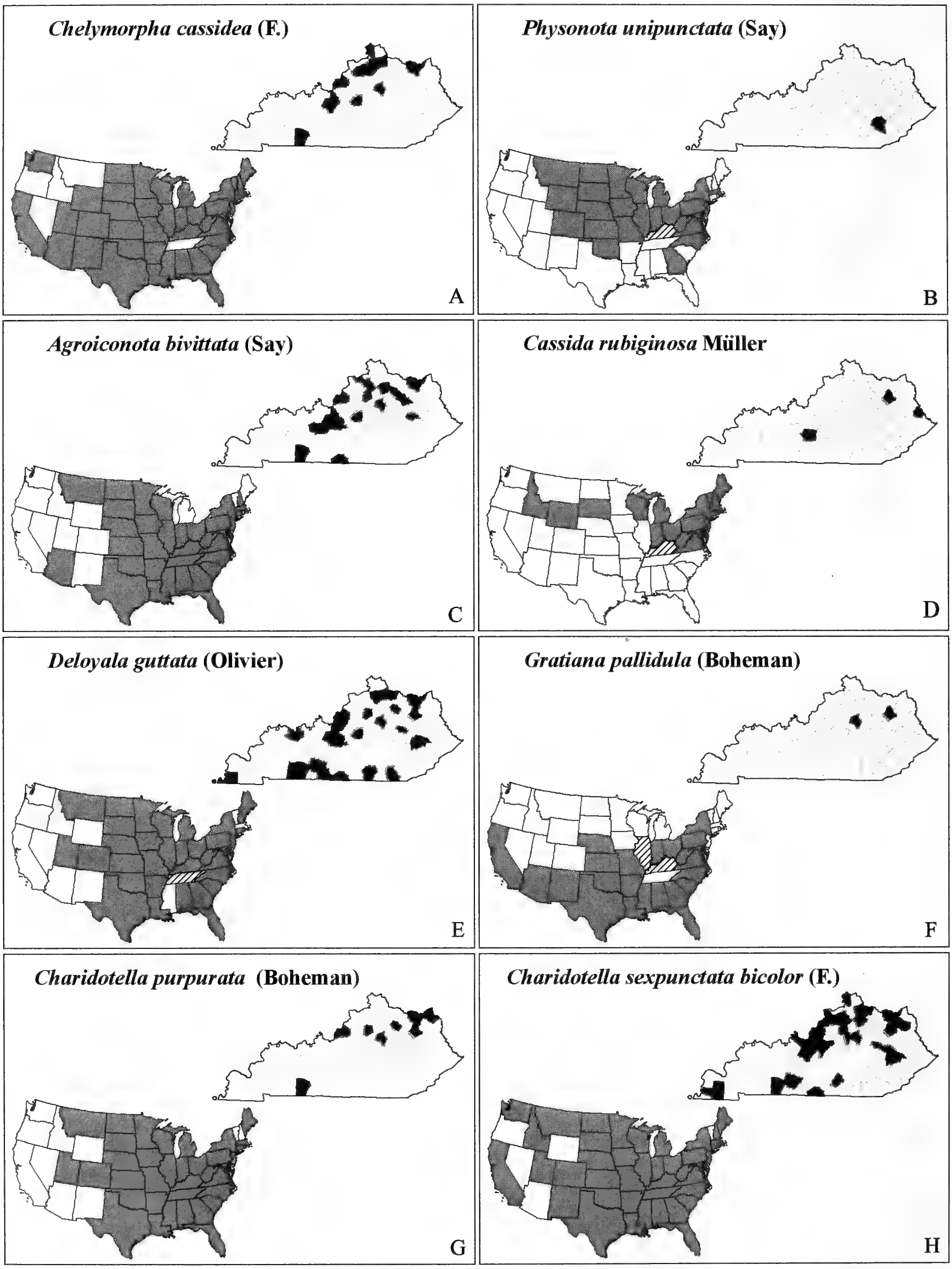

Figure 4. The known distribution of Cassidinae leaf beetles (Coleoptera: Chrysomelidae) is illustrated in grey shading in Kentucky counties and the United States. New state records reported herein are shown in cross-hatch.

Comments: This species is rare in Kentucky and not native to North America. Preferred host plants are Asteraceae (Clark et al. 2004).

Deloyala guttata (Olivier) (Figure 4E)

Kentucky Counties: Allen, Bracken, Breathitt, Bullitt, Daviess, Fayette, Franklin, Fulton, Grant, Hardin, Hickman, Jefferson, LaRue, Lewis, Logan, Monroe, Nicholas, Pendleton, Powell, Rowan, Todd, Warren, Washington, Wayne, Whitley

Years: 1891 (5), 1896 (1), 1900 (1), 1913 (1), 1933 (1), 1947 (2), 1948 (3), 1952 (1), 1955 (3), 1956 (1), 1957 (1), 1958 (3), 1960 (3), 1964 (1), 1965 (2), 1967 (2), 1968 (2), 1969 (1), 1971 (3), 1972 (2), 1974 (5), 1975 (2), 1976 (3), 1978 (1), 1980 (1), 1981 (10), 1983 (1), 1985 (1), 1995 (3), 1998 (4), 2003 (1), 2004 (9), 2005 (26), 2006 (14)

Months: March (2), April (3), May (31), June (40), July (23), August (6), September (8), October (5), November (2)

Abundance: 120 specimens: 6-BYUC, 4-CWC, 41-KSUC, 14-RJBC, 49-UKIC, 6-WKUC

Comments: The common name for this abundant species is the mottled tortoise beetle. UKIC label data reported collection by Malaise trap and association with sweet potato, *Ipomoea batatas*. A new state record was found for Tennessee in the RJBC (1 specimen from Sevier County) (Figure 4E).

Gratiana pallidula (Boheman) (Figure 4F)

Kentucky Counties: Fayette, Rowan

Years: 1895 (1), 1993 (1)

Months: May (1), July (1)

Abundance: 2 specimens: 1-CSUC, 1-UKIC (new state record)

Comments: This species is rare in Kentucky and known food plants belong to Solanaceae (Riley 1986). A UKIC label records an association with eggplant. A new state record was found for Illinois in the RJBC (1 specimen from St. Clair County) (Figure 4F).

Charidotella purpurata (Boheman) (Figure 4G)

Kentucky Counties: Fayette, Franklin, Greenup, Jefferson, Lewis, Logan, Nicholas, Rowan

Years: 1971 (1), 1976 (4), 1983 (1), 1987 (6), 1990 (3), 1992 (1), 1995 (3), 1998 (1), 2005 (1), 2006 (2)

Months: March (4), May (15), June (2), July (1), August (1)

Abundance: 23 specimens: 5-BYUC, 3-CWC, 2-KSUC, 12-RJBC, 1-UKIC

Comments: Host plants for this species are members of Convolvulaceae (Riley 1986). UKIC label data reported collection by Malaise trap.

Charidotella sexpunctata bicolor (F.) (Figure 4H)

Kentucky Counties: Boone, Bracken, Breathitt, Bullitt, Carter, Fayette, Franklin, Graves, Hardin, Harrison, Henry, Hickman, Jefferson, Lewis, Logan, Meade, Monroe, Nelson, Oldham, Owen, Pendleton, Powell, Rowan, Russell, Trimble, Warren, Wolfe, Woodford

Years: 1891 (6), 1892 (1), 1896 (1), 1897 (9), 1901 (1), 1910 (1), 1916 (1), 1934 (1), 1935 (1), 1937 (1), 1944 (5), 1946 (4), 1947 (3), 1948 (14), 1951 (7), 1953 (1), 1954 (1), 1955 (1), 1957 (4), 1958 (1), 1960 (2), 1961 (1), 1963 (1), 1965 (2), 1966 (2), 1968 (3), 1970 (2), 1971 (3), 1972 (3), 1974 (3), 1975 (6), 1976 (5), 1979 (1), 1981 (6), 1983 (5), 1984 (2), 1992 (1), 1995 (4), 1998 (2), 2003 (5), 2004 (4), 2005 (12), 2006 (6)

Months: March (1), April (1), May (47), June (55), July (20), August (6), September (6), October (8), November (1)

Abundance: 145 specimens: 7-BYUC, 10-CWC, 17-KSUC, 9-RJBC, 97-UKIC, 5-WKUC

Comments: The common name for this abundant species is the golden tortoise beetle and host plants are members of Convolvulaceae (Riley 1986). UKIC labels record associations with sweet potato, morning glory, and sassafras, but the occurrence on sassafras was almost certainly incidental. Collection by Malaise trap was also documented.

Plagiometriona clavata clavata (F.) (Figure 5A)

Kentucky Counties: Franklin, LaRue, Meade, Wolfe

Years: 1892 (1), 1935 (1), 1976 (1), 1987 (1), 1993 (1), 2000 (1), 2003 (1), 2005 (1), 2006 (1)

Months: May (2), June (1), July (3), August (2), October (1)

Abundance: 9 specimens: 3-CWC, 2-RJBC, 4-UKIC

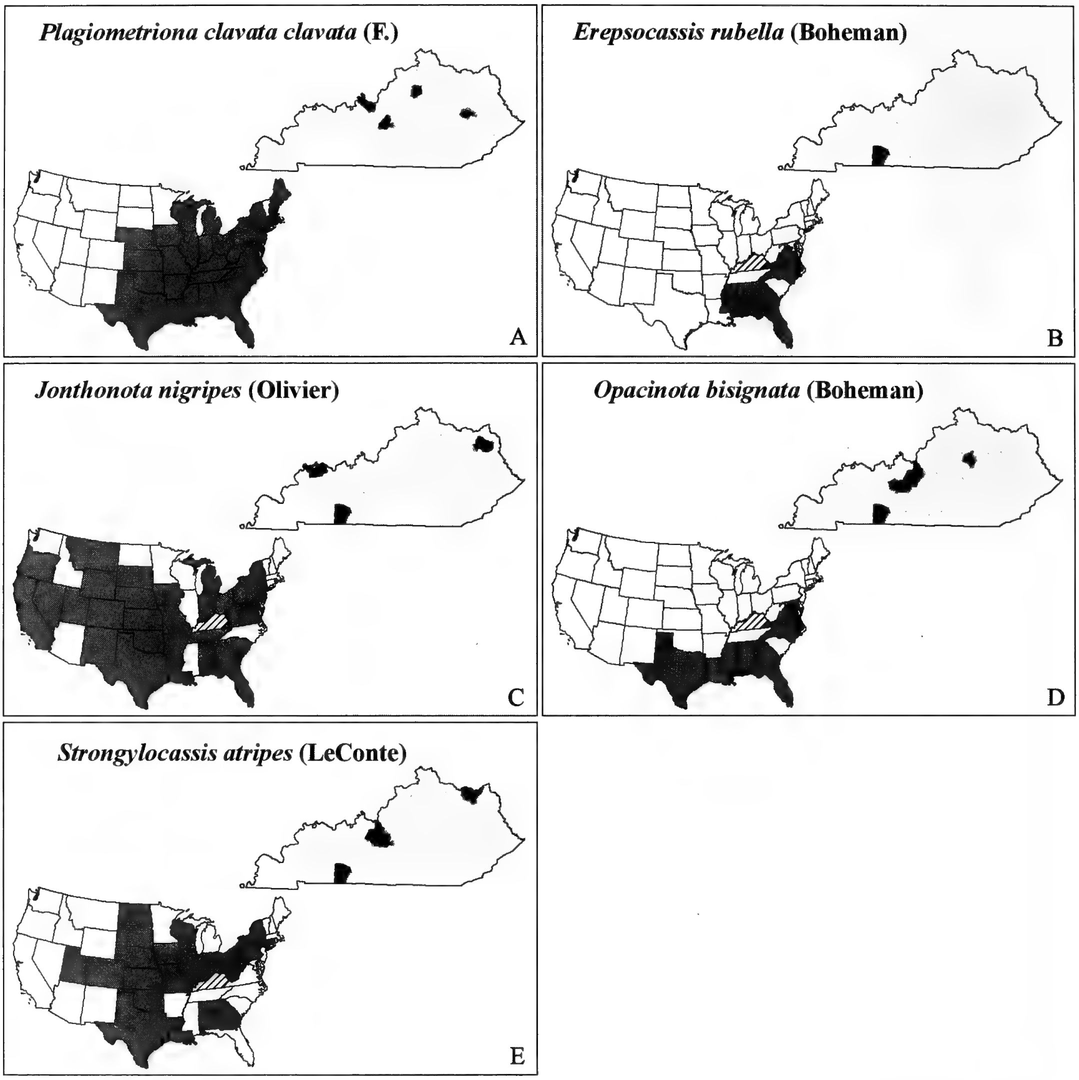

Figure 5. The known distribution of Cassidinae leaf beetles (Coleoptera: Chrysomelidae) is illustrated in grey shading in Kentucky counties and the United States. New state records reported herein are shown in cross-hatch.

Comments: This species is rarely collected in Kentucky and known food plants belong to Solanaceae (Riley 1986). UKIC label data reported collection by Malaise trap.

Erepsocassis rubella (Boheman) (Figure 5B)

Kentucky Counties: Logan

Years: 2005 (2), 2006 (14)

Months: May (1), June (8), July (6), August (1)

Abundance: 16 specimens: 16-KSUC (new state record) (Figure 5B)

Comments: All specimens from Kentucky were found at the Raymond Athey Barrens State Nature Preserve. Riley (1982) reported this species to be rare in collections. Its food plant is unknown; however, like some of the other uncommonly collected tortoise beetle species, it may utilize a relatively uncommon species of Convolvulaceae.

Jonthonota nigripes (Olivier) (Figure 5C)

Kentucky Counties: Carter, Henderson, Logan

Years: 1966 (1), 1971 (1), 2005 (2), 2006 (1)

Months: June (3), July (1), August (1)

Abundance: 5 specimens: 3-KSUC, 2-UKIC (new state record) (Figure 5C)

Comments: The common name of this species is the black-legged tortoise beetle. This species is rare in Kentucky and host plants are members of Convolvulaceae (Riley 1986). In Missouri, Louisiana and Texas the most-commonly encountered food plant is *Ipomoea pandurata* (L.) Lam.

Opacinota bisignata (Boheman) (Figure 5D)

Kentucky Counties: Fayette, Grayson, Hardin, Logan

Years: 1912 (1), 1965 (1), 1983 (1), 2004 (1), 2005 (8), 2006 (17)

Months: May (2), June (19), July (7), August (1)

Abundance: 29 specimens: 26-KSUC, 1-RJBC, 2-UKIC (new state record) (Figure 5D)

Comments: Host plants for this species are members of Convolvulaceae (Riley 1986). In Louisiana and Texas the most-commonly encountered food plant is *Ipomoea pandurata* (L.) Lam.

Strongylocassis atripes (LeConte) (Figure 5E)

Kentucky Counties: Hardin, LaRue, Lewis, Logan

Years: 1985 (1), 2004 (8), 2005 (16), 2006 (17)

Months: May (3), June (25), July (14)

Abundance: 42 specimens: 39-KSUC, 3-RJBC (new state record) (Figure 5E)

Comments: In Kentucky this species has only been found in endangered barrens. This species feeds on plants of morning glory family (Convolvulaceae) (Riley 1985a).

DISCUSSION

We believe the data presented here represent the first accurate representation of the cassidine leaf beetles known from Kentucky. The large number of new state records documented here (13 of 31, or 42% of known Kentucky Cassidinae) reflects a historical lack of leaf beetle collecting in Kentucky. Many of the early (pre-1950) specimens in the flagship collection at UKIC were collected by agricultural workers at the land-grant experiment station farm or near campus (Fayette County). This presentation of label data also permits the reader to make assessments and comparisons between species. For example, *Charidotella sexpunctata bicolor* (F.) is found in all adjacent states, 28 Kentucky counties, from 1891 to 2006, March to October, and with peak activity in May–June, and 145 specimens were recovered from all collections. This is obviously a common species. At the other end of the spectrum is *Erepsocassis rubella* (Boheman) is a new state record for Kentucky, previously known from only one adjacent state, found only at one nature preserve in 2005–2006, and active in June–July. These data imply that this is a relatively rare species, and its host plant or general habitat requirements may be met only in protected areas. Data such as these, obtained from historical and recent collections, should be of interest to the agencies that are charged with the preservation and management of natural areas.

The importance of conducting bioinventories such as this and documenting the abundance and distribution of both common and rare species can be demonstrated by another example. Three new state records were collected in 1983 by the senior author from a remnant patch of prairie along railroad tracks near Leitchfield in Grayson County, described as a 0.3 mile long patch of tallgrass prairie in the Big Barrens Region of the Mississippian Plateau (Baskin and Baskin 1977). The senior author revisited the site in 1985 and then returned 20 years later to find it mowed but still supporting some interesting chrysomelids. However, a subsequent visit in 2006 found it covered in orange traffic cones and transportation workers as part of a highway widening project - another priceless remnant of biodiversity now gone forever.

ACKNOWLEDGMENTS

Thanks are extended to Michael Sharkey and Martha Potts (UKIC), Keith Phillips (WKUC), and Charles Wright (CWC) for access to their collections. We also thank Joyce Owens (KSUC) for sorting, organizing, and transcribing, and Ken Bates (KSU GIS Research and Training Center) for creation of the distribution maps. This research was supported by USDA-CSREES Project KYX-10-05-39P.

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A Reference List to Field Botany in Kentucky (1985–2006)

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ABSTRACT

The reference list of 874 field botany entries principally from 1985 to 2006 updates two previous reference lists, the last of which appeared in 1989. Seventy-seven relevant entries through 1987 are included that were not indexed in the last reference list. One hundred fifty-two entries are from 1989 or earlier, 391 from the 1990s, and 331 since 2000. The references include 606 peer-reviewed journal articles, 67 scientific botanical books, 62 technical articles, 46 theses, 30 technical reports, 22 dissertations, 18 book chapters, and 23 popular books. The four largest botanical subjects within 11 subdivisions are floristics (247), synecology (205), autecology (134), and systematics (126).

KEY WORDS: Field botany, reference list, Kentucky

INTRODUCTION

A catalog and analysis of the literature pertaining to field botany in Kentucky is presented. Previous articles (Fuller 1979; Fuller et al. 1989) broadly addressed field botany relevant to algae, fungi, nonvascular plants, and vascular plants of Kentucky. Our compilation has the same organismal coverage, with emphasis on Kentucky peer-reviewed journal articles, relevant scientific books, theses and dissertations, book chapters, technical articles and reports from bound proceedings, conferences, and symposia that are available to the general public. We include the 12 published volumes of *The Flora of North America North of Mexico*, several taxonomic revisions and monographs with consideration of taxa in Kentucky, and significant floras, floristic lists, plant distribution works, and popular wildflower and tree books from Kentucky and contiguous states. Treatments of plant genera for southeastern United States (e.g., *Journal of the Arnold Arboreum* and *Harvard Papers in Botany*), and other continental and worldwide treatments, abstracts, newspaper articles, newsletters, popular science articles, college textbooks, and on-line articles are excluded. Weakley (2006) provides another recently compiled taxonomic bibliography with emphasis on Georgia, North Carolina, South Carolina, and Virginia.

Our objectives for this catalog and analysis of field botany in Kentucky are to provide a ready resource for researchers and others interested in the state of botanical studies in the south-central United States, and to provide helpful evidence of trends in these studies over the last two decades. These references have been gathered from numerous sources, and we have endeavored to be as comprehensive as possible. On-line search engines (e.g., AltaVista, BioOne, Google, Index to American Botanical Literature, Kew Bibliographic Database, Yahoo), greatly facilitated the preparation of the list.

RESULTS AND DISCUSSION

Fuller (1979) and Fuller et al. (1989) included 806 entries dating from 1784 to 1987. We have endeavored to cover the years from 1985 to 2006. Seventy-seven references omitted by Fuller et al. (1989) are added. Our

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Table 1. Reference sources catalogued by years 1980–1989, 1990–1999, and 2000–2006. Numbers by years and total percentages in parentheses.

Literature types	Years 1980–1989	Years 1990–1999	Years 2000–2006	Total
Refereed Journal Articles	87 (57.24)	261 (66.75)	258 (77.94)	606 (69.34)
Botanical Books	11 (7.24)	32 (8.18)	24 (7.25)	67 (7.67)
Technical Articles	25 (16.45)	29 (7.42)	8 (2.42)	62 (7.09)
MS/MA Theses	10 (6.58)	23 (5.88)	13 (3.93)	46 (5.26)
Technical Reports	12 (7.89)	15 (3.84)	3 (0.91)	30 (3.43)
Popular Books	1 (0.65)	10 (2.56)	12 (3.63)	23 (2.63)
Dissertations	6 (3.95)	10 (2.56)	6 (1.81)	22 (2.52)
Book Chapters	0 (0.00)	11 (2.81)	7 (2.11)	18 (2.06)
Totals	152	391	331	874 (100.00)

total number of entries is 874 with 152 prior to 1989, 391 from the 1990s, and 331 since 2000 (Table 1). These 874 entries are composed of 606 peer-reviewed journal articles, 67 scientific books, 62 technical articles, 30 technical reports, 46 M.S./M.A. theses, 22 Ph.D. dissertations, 18 book chapters, and 23 state and regional wildflower/tree books and field guides (Table 1).

The 874 entries were placed into 11 botanical subdivision categories during the periods of 1980–1989, 1990–1999, and 2000–2006 (Table 2). The 11 field botany subdivisions are described in the broad sense because of overlap in disciplines. Floristics (*alpha* plant taxonomy) consists of vascular plant surveys, checklists, inventories, rare and noteworthy species, floras, atlases, and popular wildflower/tree books and field guides. Phytogeography entries are combined with floristics and include native and exotic distributions and flora range extensions. Systematics (*beta* plant taxonomy) includes all biosystematic and phylogenetic studies, revisions and monographs, and new species descriptions. Synecology (community ecology) contains terrestrial and wetland vegetation studies, community dynamics (fire, soils, succession, etc.), systems ecology, and plant sociology. Autecology (ecophysiology) includes vascular plant life histories, seed germination studies, population ecology, genecology, and demography studies. Exotic (naturalized) entries pertain to the spread and control of invasives, these entries separated from the autecology, synecology, and floristic categories (e.g., several recent *Lonicera maackii* references). Paleoecology includes aspects of historical vegetation reconstruction from the geological ages. Bryology and lichenology are combined and comprised all Kentucky studies of mosses, liverworts, hornworts, and lichens; Phycology includes all studies of algae; and Mycology comprises all studies of fungi. The History category consists of the lives and works of botanists (emphasis on Kentucky individuals), historical events, and obituaries. Herbarium and natural history

Table 2. Botanical subdivisions catalogued by years 1980–1989, 1990–1999, and 2000–2006. Numbers by years and total percentages in parentheses.

Botanical subjects	Years 1980–1989	Years 1990–1999	Years 2000–2006	Total
Floristics (*a*-taxonomy)	46 (30.26)	110 (28.13)	102 (30.82)	258 (29.52)
Synecology	43 (28.29)	89 (22.76)	66 (19.94)	198 (22.65)
Autecology	17 (11.18)	80 (20.46)	48 (14.50)	145 (16.59)
Systematics (*b*-taxonomy)	23 (15.13)	50 (12.79)	56 (16.92)	129 (14.76)
Exotics	1 (0.67)	17 (4.35)	15 (4.53)	33 (3.78)
Herbaria/Natural History	4 (2.63)	7 (1.79)	16 (4.83)	27 (3.09)
History/Obituaries	2 (1.32)	4 (1.02)	19 (5.74)	25 (2.86)
Bryology	6 (3.95)	8 (2.05)	4 (1.21)	18 (2.06)
Phycology	3 (1.97)	11 (2.81)	4 (1.21)	18 (2.06)
Paleoecology	3 (1.97)	8 (2.05)	1 (0.30)	12 (1.37)
Mycology	4 (2.63)	7 (1.79)	0 (0.00)	11 (1.26)
Totals	152	391	331	874 (100.00)

Table 3. Reference entries by literature sources and subjects listed by years 1980–1989, 1990–1999, 2000–2006. Numbers by year and total percentages in parentheses.

Literature types	Years 1980–1989	Years 1990–1999	Years 2000–2006	Total References
REFEREED JOURNALS				
Floristics	25 (28.73)	57 (21.84)	59 (22.87)	141 (23.27)
Autecology	15 (17.24)	73 (27.97)	44 (17.05)	132 (21.78)
Systematics	22 (25.29)	46 (17.62)	55 (21.32)	123 (20.30)
Synecology	11 (12.64)	39 (14.94)	46 (17.83)	96 (15.84)
Exotics	0 (0.00)	16 (6.13)	15 (5.81)	31 (5.12)
Herbarium	4 (4.60)	6 (2.30)	16 (6.20)	26 (4.29)
History	0 (0.00)	3 (1.15)	17 (6.59)	20 (3.30)
Bryology	5 (5.75)	6 (2.30)	4 (1.55)	15 (2.48)
Mycology	4 (4.60)	6 (2.30)	0 (0.00)	10 (1.65)
Phycology	1 (1.15)	4 (1.53)	2 (0.76)	7 (1.15)
Paleoecology	0 (0.00)	5 (1.92)	0 (0.00)	5 (0.82)
Subtotal	87	261	258	606 (100.00)
TECHNICAL ARTICLES				
Synecology	17 (68.00)	18 (62.07)	4 (50.00)	39 (62.90)
Floristics	6 (24.00)	9 (31.03)	4 (50.00)	19 (30.65)
Bryology	1 (4.00)	1 (3.45)	0 (0.00)	2 (3.23)
Mycology	0 (0.00)	1 (3.45)	0 (0.00)	1 (1.61)
Paleoecology	1 (4.00)	0 (0.00)	0 (0.00)	1 (1.61)
Subtotal	25	29	8	62 (100.00)
TECHNICAL REPORTS				
Floristics	5 (30.00)	12 (60.00)	3 (100.00)	20 (53.13)
Synecology	7 (70.00)	3 (35.00)	0 (0.00)	10 (43.75)
Subtotal	12	15	3	30 (100.00)
BOOK CHAPTERS				
Synecology	0 (0.00)	9 (81.82)	5 (50.00)	14 (70.58)
Floristics	0 (0.00)	0 (0.00)	2 (1.67)	2 (5.88)
Paleoecology	0 (0.00)	2 (18.18)	0 (0.00)	2 (11.77)
Subtotal	0	11	7	18 (100.00)
THESES				
Synecology	4 (33.33)	12 (50.00)	9 (69.23)	25 (52.27)
Floristics	3 (33.33)	3 (13.64)	3 (23.08)	9 (20.46)
Autecology	2 (22.22)	2 (13.64)	0 (0.00)	4 (11.36)
Systematics	0 (0.00)	4 (13.64)	0 (0.00)	4 (6.82)
Phycology	0 (0.00)	1 (4.54)	1 (7.69)	2 (4.55)
Bryology	0 (0.00)	1 (4.54)	0 (0.00)	1 (2.27)
Paleoecology	1 (11.11)	0 (0.00)	0 (0.00)	1 (2.27)
Subtotal	10	23	13	46 (100.00)
DISSERTATIONS				
Synecology	4 (66.67)	4 (40.00)	2 (25.00)	10 (45.00)
Autecology	0 (0.00)	4 (40.00)	4 (75.00)	8 (35.00)
Floristics	0 (0.00)	1 (10.00)	0 (0.00)	1 (5.00)
Systematics	1 (16.66)	0 (0.00)	0 (0.00)	1 (5.00)
Phycology	0 (0.00)	1 (10.00)	0 (0.00)	1 (5.00)
Exotics	1 (16.66)	0 (0.00)	0 (0.00)	1 (5.00)
Subtotal	6	10	6	22 (100.00)
BOTANICAL BOOKS				
Floristics	6 (44.44)	18 (54.28)	19 (82.61)	43 (62.69)
Phycology	2 (22.22)	5 (14.28)	1 (4.34)	8 (11.94)
History	2 (22.22)	1 (8.57)	2 (8.70)	5 (10.45)
Synecology	0 (0.00)	4 (11.43)	0 (0.00)	4 (5.97)
Paleoecology	1 (11.11)	1 (2.86)	1 (4.34)	3 (4.48)
Autecology	0 (0.00)	1 (2.86)	0 (0.00)	1 (1.49)
Exotics	0 (0.00)	1 (2.86)	0 (0.00)	1 (1.49)
Herbarium	0 (0.00)	1 (2.86)	0 (0.00)	1 (1.49)
Systematics	0	0	1	1 (1.49)
Subtotal	11	32	24	67 (100.00)
POPULAR BOOKS				
Floristics	1 (100.00)	10 (100.00)	12 (100.00)	23

Table 3. Continued.

Literature types	Years			Total References
	1980–1989	1990–1999	2000–2006	
Subtotal	1	10	12	23 (100.00)
TOTAL	152	391	331	874 (100.00)

embrace works on Kentucky herbaria, other museum collections, and literature on the increasing importance of natural history facilities.

Designating a botanical reference to a single subdivision entry was not an easy task because of frequent overlaps. As an example, Thompson and Fleming (2004a) conducted a study of the vascular flora and plant communities of a Kentucky State Nature Preserve area in Rockcastle County. Because the focus was more on a floristic survey than quantitative synecological data, this article is placed under floristics and phytogeography. For additional information purposes, the 11 subdivisions of botany are incorporated within the eight literature types for each of the 874 entries (Table 3).

The acceleration of botanical knowledge is evident as more research is being published each year and thus each decade (Table 1). The number of publications applicable to field botany in Kentucky in the last 20 years is comparable with the total number in the previous 200 years. Most publications (606) continue to be in refereed scientific journals (69.34%). Of these journals entries (Table 3), the top four categories are floristics with 141 (23.27 %), autecology with 132 (21.78%), systematics with 123 (20.30%), and synecology with 96 (15.84%).

In refereed journal entries alone, 136 autecology, synecology, and floristic articles are authored/coauthored by Jerry and Carol Baskin, the University of Kentucky. In all literature entries, 63 are written or coauthored by Edward W. Chester, Austin Peay State University, 40 by Ralph L. Thompson, Berea College, 30 by James O. Luken, Coastal Carolina University, 24 by Julian J.N. Campbell, The Nature Conservancy, and 23 by Jeffery L. Walck, Middle Tennessee State University. The 258 peer-reviewed journal articles for the 2000–2006 period is comparable to the 261 of the 1990–1999 decade (Table 2).

A significant decline in technical articles and technical reports is noted during 2000–2006. Thirty-nine (62.90%) of 62 technical articles are in synecology and 19 (30.65%) are floristics (Table 3). The percentages of journal publications on floristics, synecology, autecology, and systematics per year have changed little since 1980 (Table 2). The 67 botanical books are comprised of 43 (64.18%) in floristics with the majority being floras, manuals, atlases, and checklists. These listings also shows an increase in studies on invasive species since 1990. Obituaries are also on the increase. The late John W. Thieret, Kentucky's foremost plant taxonomist of the 20th century, accounted for nine memoriam tribute entries.

A noticeable decline in the number of theses and dissertations in the subdivisions of field botany is occurring (Table 2). The 46 theses are from the University of Kentucky (17), Eastern Kentucky University (13), Southern Illinois University (6), Murray State University (3), University of Louisville (2), University of Tennessee (2), Morehead State University (1), Western Kentucky University (1), and Austin Peay State University (1). Twenty-five (54.35%) theses are in synecology and 9 (19.56%) are in floristics (Table 3). Of 22 dissertations, ten (45.45%) are in synecology and 8 (36.36%) in autecology (Table 3). The 22 dissertations are from the University of Kentucky (11), University of Louisville (4), Southern Illinois University (4), North Carolina State University (1), University of Tennessee (1), and University of Joensuu, Finland (1).

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Mortality and Progeny Production of Several Stored Grain Beetle Pests in Shelled Corn Treated with Entrust®

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ABSTRACT

Use of synthetic chemicals has been the primary method of insect control in stored grain. Concern for insecticide resistance, chemical residues, environmental contamination, and worker exposure has lead to development of biologically based insecticides such as Spintor® 2SC. Spintor has been found to be effective against several stored grain beetle species on wheat and shelled corn in storage. Recently it was re-formulated to conform to OMRI national standards and is now commercially available as an organic insecticide (i.e., Entrust®) registered for use on fruits and vegetables. However, no information regarding the use of Entrust on stored grains has been found. A laboratory study was conducted to determine efficacy of Entrust on maize weevil (*Sitophilus zeamais* Motsculsky), red flour beetle (*Tribolium castaneum* (Herbst)), and sawtoothed grain beetle (*Oryzaephilus surinamensis* (Linnaeus)) infesting stored shelled corn. Shelled corn was treated with 1 ppm or 3 ppm of Entrust or left untreated. Mortality was quantified at 1, 3, and 7 days after exposure. Maize weevil survival was negatively affected at all three time intervals at both the 1 ppm and 3 ppm treatments with 100% mortality observed after one week for the 3 ppm treatment. No significant differences in mortality were seen between treated and control groups of either sawtoothed grain beetle or red flour beetle. However, Entrust treated corn negatively affected progeny emergence of all three insect species.

KEY WORDS: maize weevil, sawtoothed grain beetle, red flour beetle, stored corn, organic insecticide, Entrust®

INTRODUCTION

Corn is a major crop in both Kentucky and the rest of the United States. With an average annual value of 209 million dollars between 2002 and 2006, recent reports rank Kentucky 13th among states for corn production (Kentucky Agricultural Statistics 2005–2006). After harvest approximately 50% of the crop is placed in storage. During on-farm storage corn is susceptible to damage by approximately 50 different species of insects (Sedlacek and Weston 1995). These insects are generally classified either as primary or secondary pests. Primary insect pests of stored corn are capable of attacking undamaged whole kernels. Larvae develop inside the kernels where they feed and hollow out the interior (Storey 1987). Examples include the maize weevil, *Sitophilus zeamais* Motschulsky; rice weevil, *Sitophilus oryzae* (Linnaeus); granary weevil, *Sitophilus granarius* (Linnaeus); lesser grain borer, *Rhyzopertha dominica* (Fabricius); and Angoumois grain moth, *Sitotroga cerealella* (Olivier). Secondary pests require corn that has been damaged by harvest machinery, grain handling equipment, or prior infestation by primary pests. The extent of damage influences population sizes of secondary pests. Red flour beetle, *Tribolium castaneum* (Herbst); Indianmeal moth, *Plodia interpunctella* (Hübner); rusty grain beetle, *Cryptolestes ferrugineus* (Stephens); and sawtoothed grain beetle, *Oryzaephilus surinamemsis* (Linnaeus) are some of the more common secondary pests of corn (Sedlacek and Weston 1995). Maize weevil, red flour beetle, and sawtoothed grain beetle are among the most commonly found and abundant stored corn insect pests in Kentucky (Sedlacek et al. 1998).

Damage by insect boring, feeding, and mold reduces corn quality resulting in cash discounts at market (Burkholder and Krischik 1995). This damage may result in excess of 21 million dollars in losses in Kentucky annually (Kentucky Agricultural Statistics 2005–2006). For over 50 years the most common method to control insect populations and prevent losses in stored grains in the United States has been through the use of organophosphate insecticides such as Cythion® and Actellic®.

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More recently, biologically based insecticides in the Naturalyte® insect control class composed of spinosad (e.g., Spintor® 2SC) have been developed. The active ingredients in Spintor® 2SC are fermentation byproducts derived from the naturally occurring bacterium *Saccharopolyspora spinosa*. Spinosad is a reduced risk compound whose mode of action after ingestion is characterized by excitation of the insect nervous system leading to muscle contraction and paralysis (Spintor® 2SC Specimen Label 2002). Spintor® 2SC is currently registered for use on over 200 varieties of vegetables, fruits, and grains. Recent studies have shown Spintor® 2SC to have a significant negative affect on stored grain insect pest survival and fecundity in wheat (Fang et al. 2002a, 2002b).

Public concern over environmental contamination and chemical residues on food has led to rising demand for organically produced grains. Organic meat and poultry sales showed a 78% increase in sales from 2003–2004 (Born 2005). Because diets of organically certified livestock must be organically produced and stored and due to an increased consumer demand for organic food products, average annual prices for organic grains were twice that of conventionally produced grain from 1995 to 2003 (Born 2005). Spintor® 2SC is not classified as an organic insecticide due to an inert ingredient propylene glycol (Spintor® 2SC MSDS 2001). Its recent reformulation into Entrust®, containing 80% active ingredient spinosad and 20% inert ingredient Kaolin clay/ crystalline silica, has enabled it to be registered as an organic insecticide by the USDA (Entrust® MSDS 2004). While Entrust® is also registered for use on a wide variety of vegetables, fruits, and grains; no information has been found regarding its use in stored grains.

Thus, the objective of this study was to quantify the effects of the organic insecticide, Entrust®, on mortality and progeny production of several stored grain pests in stored corn. We examined maize weevil, red flour beetle, and sawtoothed grain beetle adult mortality and progeny production.

MATERIALS AND METHODS

This research was conducted in the Insect Pest Management and Ecology Laboratory at Kentucky State University, Frankfort, KY. Insect colonies were maintained in a Percival environmental chamber set at 27 ± 1°C, ≥60% RH, in complete darkness. Maize weevils and sawtoothed grain beetles were reared in 0.9-L jars containing 0.6 L of corn or 100% rolled oats, respectively. Red flour beetles were reared in 0.9-L jars containing 0.6 L of a wheat flour/corn meal based diet plus brewer's yeast. Adults were permitted to reproduce and were removed approximately every two weeks. Colony maintenance was repeated throughout the year and insured that adults used in this experiment were between one and two weeks old.

Shelled corn at 12–14% moisture content was sorted to provide undamaged whole kernels for the maize weevils and damaged corn for red flour and sawtoothed grain beetles. Three separate treatments were prepared using the undamaged kernels. Kernels were treated with 20 mL of 1 ppm or 3 ppm Entrust® per 200 g of corn. A control treatment was prepared using 20 mL distilled water per 200 g of corn. Damaged kernels were prepared similarly using 40 mL 1 ppm Entrust®, 3 ppm Entrust®, or distilled water per 400 g of corn for each treatment to be used for the two secondary pests. Treated kernels in jars were placed on a roller mill for two min to uniformly coat and then placed aside to dry for at least 48 hr.

Fifteen ventilated vials of each treatment for each species were filled with 11.0 g of either undamaged kernels for the 45 vials used for the maize weevils or damaged corn for the 90 vials used for the two secondary pests. All vials were labeled and placed in three crispers using a completely randomized block design. Maize weevils were sexed with 6 females and 3 males introduced per vial. Ten sawtoothed grain beetles and 10 red flour beetles were not sexed and were introduced into their respective vials. Vials were immediately placed in a Percival environmental chamber set at 27 ± 1°C, ≥60% RH, in complete darkness.

Adult mortality was quantified and recorded at 1, 3, and 7 days after beetle introduction. All adults were removed after the one week mortality check. Checks for progeny emergence, as F_1 adults, were conducted daily starting 3 weeks after exposure and continued for 6 weeks. Vials were kept

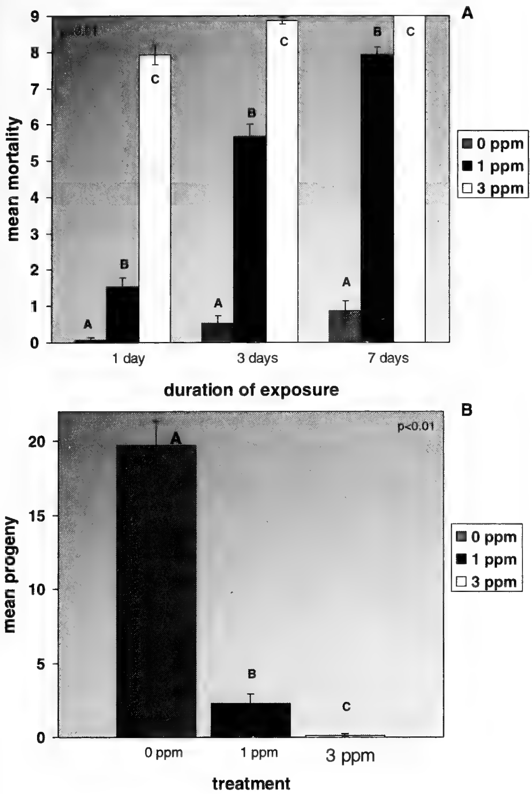

Figure 1. Maize weevil adult mortality (A) and progeny production (B) after 1, 3, and 7 day exposures to shelled corn treated with Entrust at 0, 1, and 3 ppm.

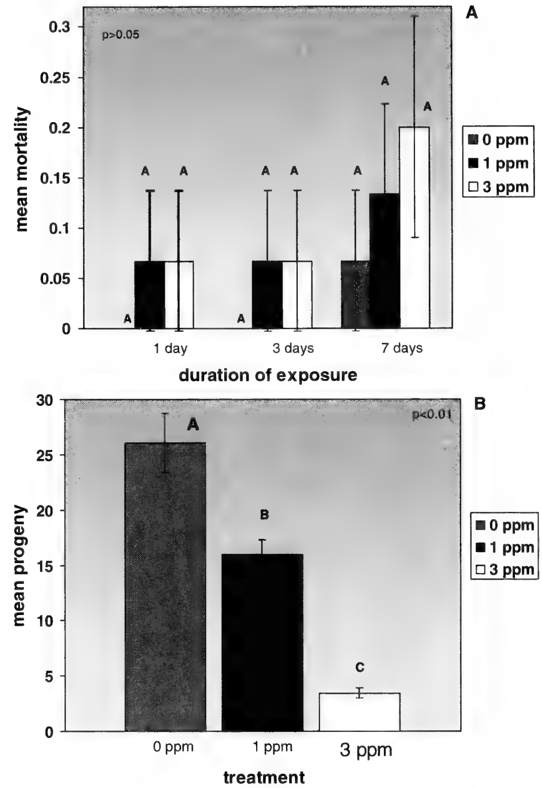

Figure 2. Red flour beetle mortality (A) and progeny production (B) after 1, 3 and 7 day exposures to shelled corn treated with Entrust at 0, 1, and 3 ppm.

inside the environmental chamber unless being checked. Mortality and progeny data were analyzed with SAS using a repeated measures ANOVA and LSD procedures at $P \leq 0.05$ (SAS Institute 2003).

RESULTS AND DISCUSSION

Maize weevil survival was significantly negatively impacted ($P < 0.01$) at all three time intervals at both the 1 ppm and 3 ppm treatments of Entrust®, with 100% mortality observed after one week for the 3 ppm treatment (Figure 1A). No significant differences were seen at any time interval for any treatment of either red flour beetle or sawtoothed grain beetle mortality (Figure 2A, Figure 3A). Any impact of Entrust on red flour and sawtoothed grain beetle adult populations was minimal considering average mortality was less than 0.5 at 1 and 3 ppm treatments. Average progeny emergence for all three insect species was significantly lower than controls at 1 ppm, and emergence in the 3 ppm treatment was significantly reduced compared with the 1 ppm treatments ($P < 0.01$) (Figures 1B, 2B, 3B).

While stored corn treated with 1 ppm or 3 ppm Entrust® showed significant negative impact on maize weevil survival and progeny emergence, only progeny emergence was shown to be negatively effected in vials containing sawtoothed grain or red flour beetles. Results were interesting when compared with a previous study conducted at Kentucky State University examining shelled corn treated with 1 ppm Spintor® 2SC (22.8% a.i.). One ppm Spintor® 2SC caused 70%, 24%, and 16% mortality of maize weevils, red flour beetles, and sawtoothed grain beetles, respectively (Sedlacek, unpublished data). One ppm Entrust® (80% a.i.) caused 88%, 1.3%, and 2.7% mortality of the same three beetles, respectively. Progeny emergence was reduced 63%, 100%, and 72%, respectively, for maize weevils, red flour beetles and

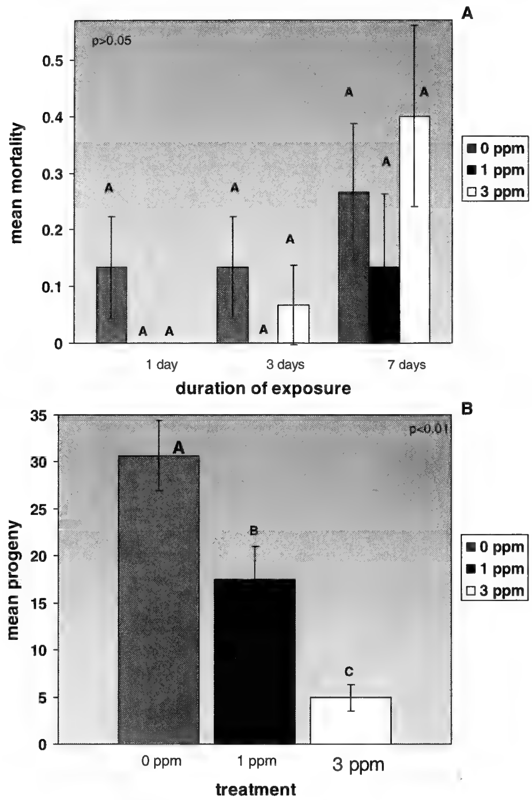

Figure 3. Sawtoothed grain beetle mortality (A) and progeny production (B) after 1, 3 and 7 day exposures to shelled corn treated with Entrust at 0, 1, and 3 ppm.

sawtoothed grain beetles in the 1 ppm Spintor experiment (Sedlacek, unpublished data). Emergence was reduced 88.5%, 38.8%, and 43%, respectively when using 1 ppm Entrust treated corn.

Based on the results of the experiment presented herein, Entrust does not appear to be as effective as Spintor 2SC when applied at 1 ppm concentration for controlling populations of sawtoothed grain beetle or red flour beetle; however, Entrust appeared to have a greater effect on maize weevil populations than Spintor 2SC at the 1 ppm concentration. Similar results were found for rice weevil, *Sitophilus oryzae* (Linnaeus), red flour beetle, and sawtoothed grain beetle adult mortality and progeny production on Spintor treated durum wheat (Fang et al. 2002a, 2002b). At 3 ppm, Entrust showed greater than 80% progeny reduction of all three beetle species. It is unclear whether or not reduced progeny emergence for any of the species is due to a sublethal effect on adult beetles causing reduced fecundity or on actual mortality of eggs and developing F_1 larvae. Other possible explanations for these results may be due to the differences in inert ingredients which were changed during the re-formulation process.

Using Entrust at the higher concentration may be cost effective for organically grown and stored grains. Enterprise budget analyses need to be performed to determine the economic feasibility of using this product at this rate on stored corn or other grains. Further experiments are needed to examine effects of Entrust on a greater array of stored grain insect pest and beneficial insects at low, intermediate and high concentrations. In addition, field scale assays should be conducted to determine impact on field populations in grain bins.

ACKNOWLEDGMENTS

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Polynuclear Aromatic Hydrocarbons in Sediments and Mussel Tissue from the Lower Tennessee River and Kentucky Lake

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ABSTRACT

Sediment and freshwater mussel tissues were used to evaluate distribution and bioaccumulation of polynuclear (polycyclic) aromatic hydrocarbon (PAH) compounds in the lowermost Tennessee River and Kentucky Lake. The target analytes included naphthalene, phenanthrene, anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benz[a]pyrene, and benzo[g,h,i]perylene. Surface sediments (0–5 cm depth) from four locations in lowermost Tennessee River and five locations in Kentucky Lake were analyzed for target PAH compounds to determine spatial distributions. A sediment core from Ledbetter embayment in Kentucky Lake was analyzed to describe vertical distributions of the compounds. Freshwater mussels (Unionidae) collected from the lowermost Tennessee River and Kentucky Lake were analyzed and examined for bioaccumulation. PAH compounds were detected in all sediments and mussel tissues. PAH concentrations ranged from 0.01 (detection limit) to 90.4 ng/g dry wt in sediments and 0.02 (detection limit) to 223 ng/g dry wt in mussel tissue. Considering spatial distributions of PAHs in the sampling sites, there was no clear difference found between Kentucky Lake and the lowermost Tennessee River. Accumulation patterns of PAH species in sediment and mussel tissues exhibited the following order: Naphthalene > Phenanthrene > Benz[a]pyrene > Anthracene > Benzo[b]fluoranthene > Benzo[k]fluoranthrene > Benzo[g,h;i]perylene. The results revealed that 66.2% (w/w) were non-carcinogenic PAHs, whereas 33.8% (w/w) were carcinogenic in Kentucky Lake and the lowermost Tennessee River. In comparison with other freshwater ecosystems, PAH concentrations in Kentucky Lake and the lowermost Tennessee River were low.

KEY WORDS: PAHs, sediments, mussels, lower Tennessee River, Kentucky Lake

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are hydrophobic organic contaminants and identified by the United States Environmental Protection Agency (USEPA) as priority environmental pollutants (EMAP 1982; Mumtaz and George 1995). PAHs are formed from the incomplete burning of coal, oil, gas, wood, garbage, or other organic substance and result from both anthropogenic and industrial activities (Bzdusek and Christensen 2004; Lee et al. 2004; Ohura et al. 2004a, 2004b). The release of PAHs into the environment through human activities continues to increase (Brenner et al. 2002; Booiji et al. 2003; Fernandez et al. 2003; Hafner et al. 2005). PAHs can enter surface water through deposition of airborne PAHs, discharge of municipal waste water, runoff from urban storm water and coal storage areas, wood treatment plants and other industries, oil spills, and petroleum processing (Bzdusek and Christensen 2004; Ohura et al. 2005; Chen et al. 2006). Because PAHs are non-polar, hydrophobic, and relatively stable, they tend to accumulate in environmental and biological matrices and elicit toxic effects (Carpenter et al. 2002; Cornellissen et al. 2006).

Environmental contamination by PAHs has become a great concern due to their distribution in water and sediment and bioaccumulation in terrestrial environments as well as in aquatic plants, fish, and invertebrates (Farmer et al. 2003; Metre and Mahler 2004; Nakata et al. 2003; Zakaria et al. 2002). Although a large volume of literature is available on the levels of PAHs in sediments and aquatic organisms from natural lakes, coastal and oceanic environments, very limited information is available on the PAH levels in man-made reservoirs or lakes (Maruya et al. 1997; Metre and Mahler 2004; Naumova 2002; Vinturella et al. 2004; Zhang et al. 2005).

Kentucky Lake is the largest man-made lake in the southeastern United States (Fig-

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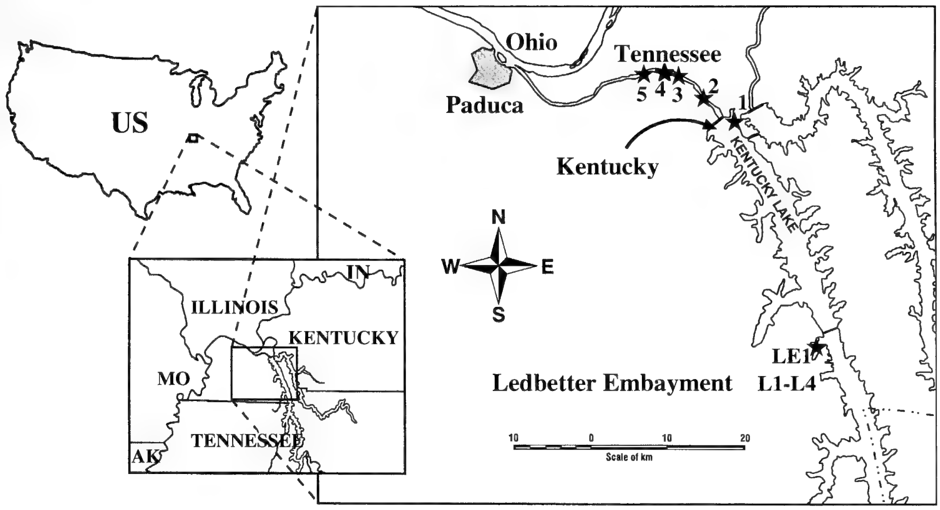

Figure 1. Map showing sediments and mussel sampling locations. Site 1: Tennessee River Mile 23.1 (Kentucky Lake mooring); Site 2: Tennessee River Mile 20.9 (I-24 Bridge). Site 3: Tennessee River Mile 17.7 (Near Air Product outfall). Site 4: Tennessee River Mile 15.2 (Near Atofina outfall). Site 5: Tennessee River Mile 10.1 (the mouth of Cypress Creek) and Ledbetter Embayment sites: L1, L2, L3, L4.

ure 1). It was created and used for multiple purposes, including generation of hydroelectric power, flood control, water supply, recreation, and transportation. Very limited information is available on the contamination levels and bioaccumulation of organic contaminants including PAHs in this Kentucky Lake ecosystem. The present study was conducted to describe the levels and accumulation pattern of PAHs in sediment and mussel tissues collected from Kentucky Lake and the lowermost Tennessee River.

EXPERIMENTAL METHODS

Field Sampling

The sampling sites included, Kentucky Lake mooring site (TRM 23.1), I-24 Bridge (TRM 20.9), near the Air Products outfall (TRM 17.7), near the Atofina outfall (TRM 15.2), Cypress Creek mouth (TRM 10.1), and Ledbetter Embayment (Figure 1; Tables 1, 2). The Kentucky Lake mooring site is used for parking large barges. The Ledbetter Embayment site was located away from the mainstream of Kentucky Lake; therefore, the sediments in the embayment were expected to be less disturbed. The sediments of these sites were analyzed to describe vertical distributions of PAHs and to reveal the historical record of the accumulation of these compounds. The surface sediments (top 0–5 cm section) from the above sites were expected to represent recent inputs of PAHs at each sites. PAHs data from these sites were used to describe spatial variation of PAHs in Kentucky Lake and the lowermost Tennessee River (Kentucky Dam Tailwater).

Surface sediments (0–5 cm) and mussel samples (grab samples) were collected by SCUBA diving. Four sediment samples from Ledbetter Embayment and five sediment samples from the lowermost Tennessee River were collected. Sediments were stored in pre-cleaned I-Chem glass bottles and kept at −20°C until further analysis. The mussel samples were identified to species. The ages of the mussels were determined by counting shell growth rings. The mussel tissues were separated from shells, and tissues of the same age and species that were collected from the same sampling site were pooled and transferred into pre-cleaned I-Chem bottles. A 40 cm long sediment core was collected at Ledbetter (LE) site using a custom-made iron core sampler equipped with a stainless steel inner-liner (length: 90 cm, internal diameter: 7.5 cm). The core was cut into 2.5 cm sections using pre-cleaned stainless steel knife and each section was transfer to a pre-cleaned wide mouth I-Chem glass bottles and kept at −20°C until further analysis. All samples were

Table 1. Details of the sampling locations and dates of sampling.

Site No.	Location	Date of sampling	Latitude	Longtitude
S1	TRM 23.1 (KY Lake Mooring)	5/10/2004	N37° 00′ 17.7″	W88° 14′ 57.5″
S2	TRM 20.9 (I-24 Bridge)	5/10/2004	N37° 01′ 46.7″	W88° 17′ 18.7″
S3	TRM 17.7 (Near Air Product Outfall)	5/10/2004	N37° 03′ 22.3″	W88° 20′ 01.6″
S4	TRM 15.2 (Near Atofina Outfall)	5/10/2004	N37° 03′ 27.4″	W88° 22′ 43.8″
S5	TRM 10.1 (Cypress Creek Mouth)	5/10/2004	N37° 01′ 48.4″	W88° 27′ 11.9″
LE1	TRM 41.7 (Ledbetter Embayment)	10/22/2004	N36° 45′ 02.0″	W88° 08′ 19.0″
L1	TRM 42.2 (Ledbetter Embayment)	11/23/2004	N36° 44′ 31.1″	W88° 08′ 39.5″
L2	TRM 42.3 (Ledbetter Embayment)	11/23/2004	N36° 44′ 25.0″	W88° 08′ 40.0″
L3	TRM 42.3 (Ledbetter Embayment)	11/23/2004	N36° 44′ 25.8″	W88° 08′ 36.3″
L4	TRM 42.2 (Ledbetter Embayment)	11/23/2004	N36° 44′ 30.9″	W88° 08′ 34.8″

freeze dried for 60 h using a Freezone Freeze Dry System (Model: 77535) and stored at 4°C until analysis.

ANALYTICAL PROCEDURE

PAHs were analyzed following the procedure describe by EMAP (Environmental Monitoring and Assessment Program 1982) and Maruya et al. (1997). About 20 g of freeze dried sediment samples were Soxhelt extracted using 325 mL of a 3:1 v/v ratio of dichloromethane/hexane (pesticide grade, Fisher Scientific/ optima, Fisher Scientific) mixture for 16 hours. The extract was concentrated to 10 mL using a Rapid Vap Evaporation system (Labconco Model 7910000). Analytes were transferred to hexane by repeating Rapid Vap concentration twice after adding 100 mL of hexane (optima, Fisher Scienfic) each time. The sample extract was further concentrated to 5 mL using a stream of ultra high purity nitrogen gas to evaporate the solvent. To separate the PAH compounds, silica-gel column chromatography was carried out to remove interfering organic and polar species. 1.5 g of Silica gel (Wakogel® S-1, Wako Pure Chemicals Industries, Japan) was activated by heating it at 130°C in an oven for 3 h. The silica gel was immediately added to 20 mL of ultra pure hexane (B&J GC²®, Burdick & Jackson, USA) and packed in a 10 mm i.d. glass column. About 3 g of anhydrous sodium sulfate (certified A.C.S., 10–60 mesh, Fisher Scientific) was then added on top of the silica gel to remove any water that might have been in the sample. The sample extract was loaded on the column and eluted with solvents. Two different fractions, F1 and F2, were taken from the column. The first fraction (F1) containing low molecular weight PAHs was eluted with 120 mL of ultra pure hexane. The second fraction (F2) containing most of the high molecular weight PAH compounds was eluted with 150 mL (50% v/v) dichloromethane/ hexane. F1 was concentrated using a Rapid Vap concentration apparatus to 10 mL followed by nitrogen gas evaporation to 0.1 mL and then analyzed using High Performance Liquid Chromatography with Fluorescence Detector (HPLC-FD). F2 also was concentrated 0.1 mL similar to F1. Freshly activated copper (granular, 99%; Lancaster) was used to remove elemental sulfur in the sample extract. The extract was analyzed using HPLC-FD. To analyze PAH compounds in mussel tissue, 5 g of freeze dried sample was extracted and purified in the same manner as for sediment samples. However, after Soxhelt extraction, lipids were removed from the tissue sample

Table 2. Details of mussel samples collected from the lowermost Tennessee River and Kentucky Lake.

Site No.	Common name	Scientific name	Date of sampling	Age (years)
S1	Asian clam (5)*	*Corbicula fluminea* (Müller)	5/10/2004	5
S2	Purple wartyback (1)	*Cyclonaias tuberculata* (Ranfinesque)	5/10/2004	16
S3	Threeridge (7)	*Amblema plicata* (Say)	5/10/2004	14–16
S4	Threeridge (5)	*Amblema plicata* (Say)	5/10/2004	15–16
S5	Pink heelsplitter (1)	*Potamilus alatus* (Say)	5/10/2004	15
S5	Yellow sandshell (1)	*Lampsilis teres* (Rafinesque)	5/10/2004	15

* Values in parenthesis indicate number of specimens pooled for analysis.

using Florisil column chromatography. For each sample, 20.0 g of Florisil (60–100 mesh, Fisher Scientific, PA, USA) was packed into a 25 mm i.d. glass column. The extracted sample was then added into the column. A gentle stream of nitrogen gas was passed through the column to remove hexane. Then, 150 mL of a 4:1 v/v ratio of acetonitrile/nanopure water (pesticide grade, Fisher Scientific/deionized water washed with hexane) mixture was passed through the column. The eluate was collected on 100 mL of hexane (Optima, Fisher Scientific) in 600 mL of nanopure water in a separatory funnel. The mixture was shaken for 15 min. After removing all lower water layer, the sample extract was washed twice with 100 ml of nanopure water for 15 minutes. The organic layer was separated and then reduced to a 5 mL volume using Rapid Vap system. After lipid removal, the extract was further cleaned using silica gel column chromatography in the same manner as sediment samples. PAH compounds were analyzed using a Shimadzu HPLC (Model: SCL-10A VP) system interfaced with a Shimadzu auto-injector (Model: SIL-10AD VP). The HPLC was equipped with fluorescence detector (Model: RF-10AXL). The column (Prevail® C18, 5 μm, 150 × 4.6 mm) condition program began Isocratic elution for 4 min using acetonitrile/water (4:6) (v/v) at 1.5 mL/min flow rate, then linear gradient elution to 100% acetonitrile over 27 min at 0.8 mL/min flow rate. Seven different PAHs (Naphthalene, Phenanthrene, Anthracene, Benzo[b]fluoranthene, Benzo[k]-fluoranthene, Benz[a]pyrene, Benzo[g,h,i]-perylene) were quantified in the samples. To determine retention times of the individual PAHs, pure standards were injected into the HPLC-FD. The retention times obtained were used to identify the PAHs in the standard mixtures. Five different concentrations of the standard mixture were injected in order to obtain calibration curves of the target PAHs. The mean slope (response factors) and r^2 values were calculated for individual PAHs. The PAHs were identified in the sample extract by comparing the retention time from the standard mixture and quantified using the response factors.

Quality assurance and quality control protocols were followed to evaluate the reliability of the data. The approved method was used to calculate the detection limits. The area of baseline noise over the elution time of each PAH compound was determined from seven injections of the matrix blank spiked with the lowest concentration of the calibration standard. The limit of detection (LOD) and limit of quantitation (LOQ) were three times and ten times the standard deviation of the baseline noise respectively divided by the slope of the calibration curve. Reagent blank was used to check laboratory contamination. The concentration of analytes detected in the reagent blank was less than the method detection limit. Also the relative accuracy of the method was determined using Standard Reference Material-1941b and 1974a in which 100 ± 30% of the known certified material was obtained. Calibration and calibration verification (five-point calibration with $r^2 = 0.995$) were checked routinely.

RESULTS

Spatial Distribution of PAHs in the lowermost Tennessee River and Kentucky Lake

Total PAHs data presented in this study is the sum of seven PAHs measured. Among the surface sediment samples (Figure 2), the sample from the mooring site in Kentucky Lake (TRM 23.1) exhibited the highest total PAH concentration (263 ng/g dry wt), followed by samples collected near I-24 Bridge or TRM 10.1 (99.3 ng/g dry wt). The lowest total PAH concentration (19.9 ng/g of dry wt) was found in sample from the Ledbetter site L1.

Among the various PAHs measured, naphthalene and phenanthrene were found in most of the surface sediment samples from the lowermost Tennessee River and Kentucky Lake (Figure 3). However, benzo[g,h,i]perylene was found to have the highest concentration at Ledbetter site L4 in Kentucky Lake (Figure 3). Naphthalene concentrations ranged from below detection limit (BDL) (<1.72 ng/g dry wt) to 38.7 ng/g dry wt. Naphthalene also was found to have the highest concentration at the mooring site in Kentucky Lake followed by sediments collected from the area around the Air Product outfall (TRM 17.7). Phenanthrene concentra-

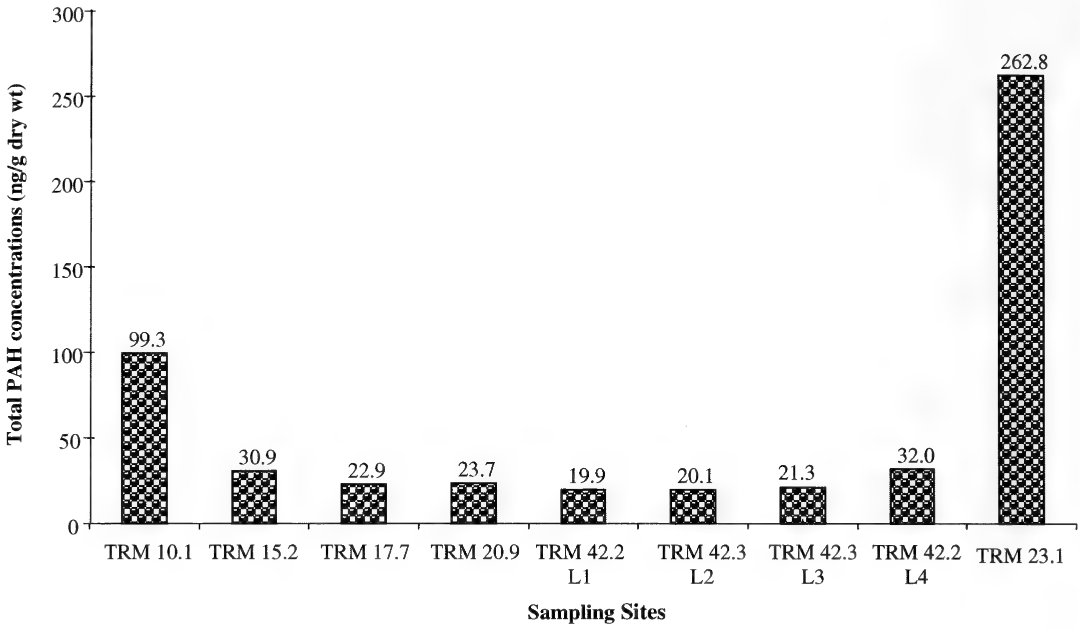

Figure 2. Total PAH concentrations (ng/g dry wt.) in surface sediment samples collected from the lowermost Tennessee River and Kentucky Lake.

tions ranged from 3.21 to 90.4 ng/g dry wt. The highest concentration (90.4 ng/g dry wt) of phenanthrene was found in sediments collected at the mooring site in Kentucky Lake, followed by a sample collected near the I-24 Bridge in the lowermost Tennessee River (11.4 ng/g dry wt). Anthracene was found in relatively low concentrations compared with phenanthrene. Anthracene concentrations ranged from below detection limit (<0.24 ng/g dry wt) to 6.84 ng/g dry wt. The highest anthracene concentration was found in sediment collected at the mooring site (Site 2 on Figure 1) in Kentucky Lake. Anthracene made up about 2% (w/w) of total PAHs in surface sediments, while benzo[b]fluoranthene, benzo[k]fluoranthene, benz[a]pyrene and benzo[g,h,i]perylene made up about 17%, 5%, 11% and 14% (w/w), respectively, of the samples. Benzo[g,h,i]perylene was BDL in sediment from the Ledbetter site L2. The highest concentration of all individual PAHs was found in samples from the mooring site (Figure 3) in Kentucky Lake. Surface sediment from the mouth of Cypress Creek also was found to contain relatively higher level of PAHS than samples from other sites in the lowermost Tennessee River.

Vertical Distribution of PAHs in Kentucky Lake and the lowermost Tennessee River

Total PAH concentrations ranged from 5.62 ng/g to 57.5 ng/g dry wt (Figure 4). PAH concentrations showed relatively higher concentrations of PAHs at the 0 to 5.0 cm depth. Comparatively higher levels of total PAHs were found in surface sediments with a gradual decreasing concentration with depth up to 7.5 cm, then in gradually increasing concentrations from to 7.5 to 12.5 cm. Relatively low and stable concentrations of total PAHs were found at 12.5 to 22.5 cm and then no clear trend was observed for deeper sediment. Naphthalene and phenanthrene were the predominate species among the PAH compounds. Anthracene was found throughout the core with relatively low concentrations at depths of 7.5 to 10 cm, 22.5 to 25 cm, 27.5 to 35 cm and 37.5 to 42.5 cm. Anthracene also was found in the core with below the detection limit on the surface and at 10 to 22.5 cm and 35 to 37.5 cm deep. The highest PAH concentration of 57.5 ng/g dry wt was found at 40 to 42.5 cm. Anthracene and benzo[b]fluoranthene concentration were below detection limits in more than 50% of the samples. Benzo[k]fluoranthene, benz[a]-

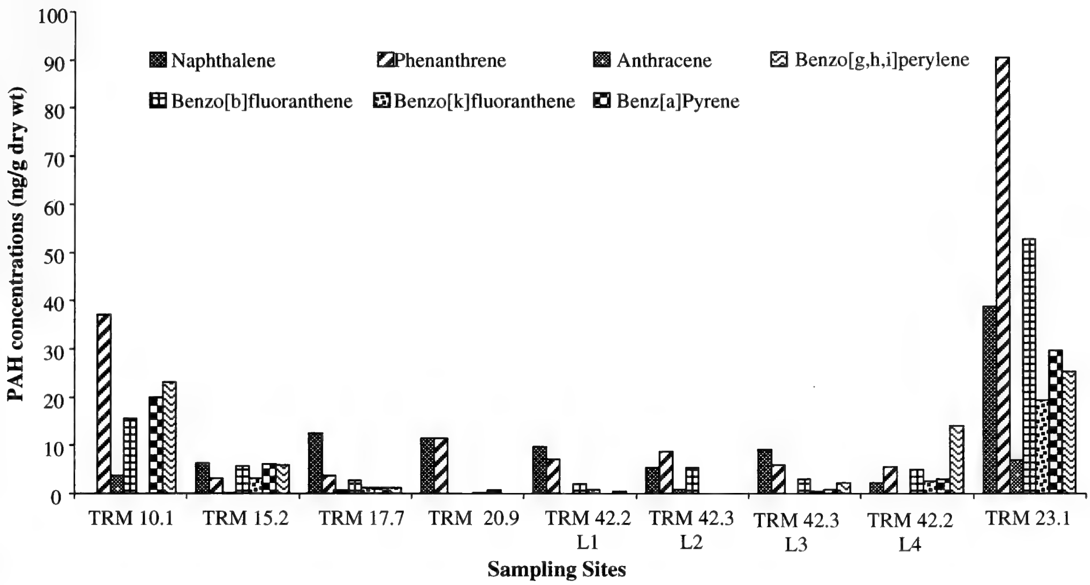

Figure 3. PAH concentrations in surface sediments from the lowermost Tennessee River and Kentucky Lake.

pyrene and benzo[g,h,i]perylene compounds were detected in the highest concentrations in the 40 to 42.5 cm section of the core sediment and contained 4.37 ng/g, 5.26 ng/g and 10.2 ng/g dry wt respectively.

Total PAH concentrations found in mussel samples ranged from 90.1 to 340.9 ng/g dry wt (Figure 5). Total PAH concentrations (340.9 ng/g dry wt) were highest in the pink heelsplitter (*Potamilus alatus*) and yellow

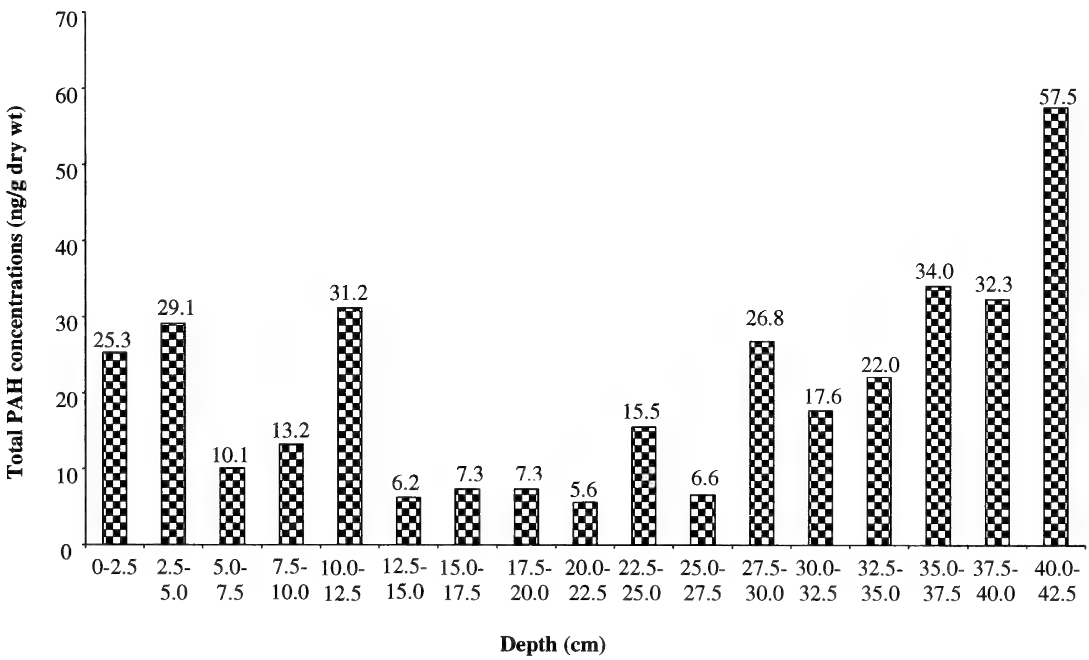

Figure 4. Total PAH concentrations (ng/g dry wt) in various sections of a sediment core collected from Ledbetter Embayment in Kentucky Lake.

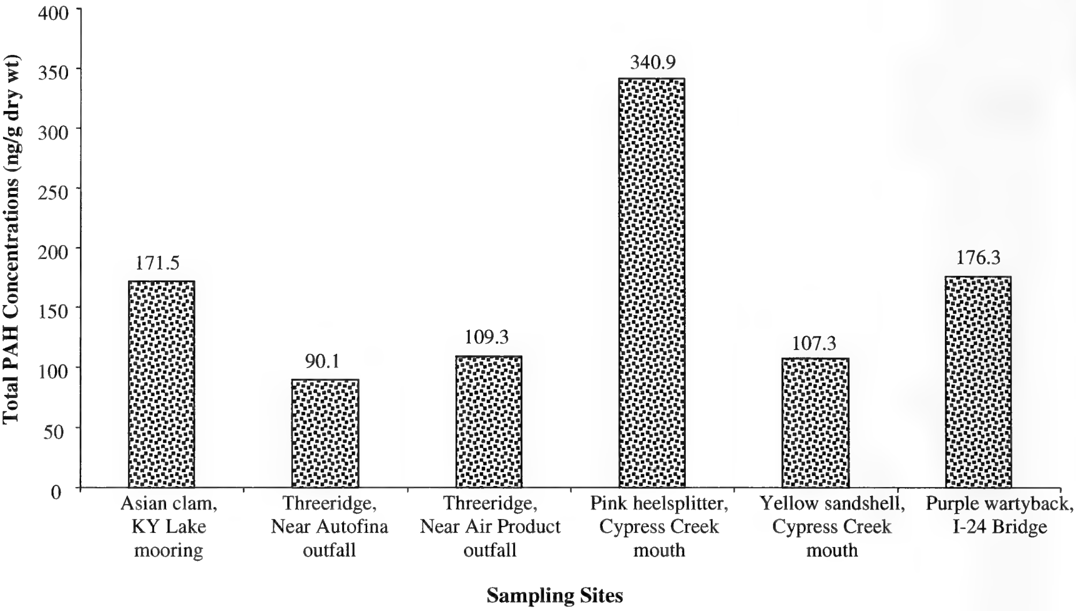

Figure 5. Total PAH concentrations (ng/g dry wt.) in mussel tissue samples collected from the lowermost Tennessee River and Kentucky Lake.

sandshell (*Lampsilis teres*) collected from mouth of Cypress Creek in the lowermost Tennessee River, followed by purple wartyback (*Cyclonaias tuberculata*) collected near the I-24 Bridge and Asiatic clam (*Corbicula fluminea*) collected at the mooring site in Kentucky Lake. Total PAH concentrations were 176.3 ng/g dry wt and 171.5 ng/g dry wt respectively. Naphthalene concentrations were relatively higher than other target compounds. Naphthalene concentrations ranged from 10.9 ng/g dry wt to 86.4 ng/g dry wt. The highest naphthalene concentration was found in a 15 year old pink heelsplitter collected from mouth of Cypress Creek in the lowermost Tennessee River. Benzo[g,h,i]-perylene was found below the detection limit (<0.02 ng/g dry wt.) or barely detected, with the exception of a purple wartyback collected from the area at the I-24 Bridge with 1.91 ng/g dry wt.

DISCUSSION

Spatial Distribution

Because of the large amount of PAHs released into the environment, determining concentrations of PAHs in the environmental and biological media is essential for understanding sources, methods of transport, and potential negative health effects. PAHs in the atmosphere are transported by wet and dry deposition into soil, water, and vegetation. When PAHs are released into soil, they can volatilize, photolyze, oxidize, biodegrade, accumulate in plants, or enter groundwater (Wick et al. 2004; Zimmerman et al. 2004). Similarly, various chemical processes can occur with PAHs in surface water including volatilization, photolysis, oxidization, biodegradation, binding to suspended particles or sediments, and bioaccumulation in aquatic organisms (Zakaria et al. 2002; Brun et al. 2004; Marr et al. 2004).

Analysis of PAHs in the lowermost Tennessee River and Kentucky Lake is helpful determining the baseline concentrations of contamination, in order to understand the inputs, bioaccumulation in these freshwater ecosystems. Although PCBs and chlorinated pesticides have been reported in Kentucky Lake sediments and mussel tissue, very limited information is available on the PAH contamination in the sediment and biota (Loganathan et al. 2001). The present study revealed the presence of seven PAH compounds in all of the sediments collected. However, the spatial distribution showed no

clear concentration patterns. The concentration of total PAHs in Kentucky Lake mooring site was relatively higher than the PAH concentrations found in the sediments from the lowermost Tennessee River. The mooring site (TRM 23.1) in Kentucky Lake is located near the dam and used for parking large barges. This site with large amount of sediment depositions has been found to have relatively high concentration of PAHs (99.3 ng/g dry wt) compared with other sampling sites in the lowermost Tennessee River (TRM 20.9, TRM 17.7 and TRM 15.5). Among the PAHs measured, pheanthrene was found to be the predominant analyte (Figure 3). Phenanthrene was found in all of sediment samples. The lowest concentration of PAH species (anthracene) was found to range from below detection limit to 6.84 ng/g dry wt. The concentration of total PAHs in sediments in these study areas are comparatively lower than several other freshwater ecosystems. The reported concentration ranged from 15,000 to 120,000 ng/g dry wt in Central Park Lake in New York City and in upper Mystic Cake in the Boston area respectively (Metre and Mahler 2004; Yan et al. 2005). Those studies analyzed 11–16 different PAHs, whereas in the present study only 7 PAHs were measured.

Transport and partitioning of PAHs in the environment also are correlated with their molecular weights (Hafner and Hites 2003). The target PAHs are divided into 2 categories, low molecular weight compounds (152–178 g/mol) and high molecular weight compounds (228–278 g/mol) (Jacob 1996; Norramit et al. 2005). In this study, PAH concentrations have been detected as low molecular weight compounds containing two or three ringed PAHs (280.2 ng/g dry wt) and high molecular weight PAHs containing five or six ringed (252.7 ng/g dry wt). Our study revealed that the amount of low molecular weight PAHs were not significantly different compared with those of high molecular weight PAHs.

Several target PAH compounds are known as human carcinogens and are classified into two categories depending on their carcinogenicity. The carcinogenic PAHs are benzo[b]fluoranthene, benzo[k]fluoranthene, and benzo[a]pyrene. Non-carcinogenic PAHs are naphthalene, anthracene, phenanthrene, and benzo[g,h,i]perylene (Norramit et al. 2005). In this study, it was shown that 33.8% (w/w) of the total PAHs found in the study sites (both Kentucky Lake and lowermost Tennessee River) were carcinogenic PAHs, whereas 66.2% (w/w) were non-carcinogenic PAHs.

Vertical Distribution

A sediment core, represents a record of contaminant inputs in a freshwater system reflecting a continuous sequence of sediment and an accumulation of PAHs over time. Therefore, sediment cores can be used to estimate the history of pollutant input to the aquatic ecosystem (Metre and Mahler 2004). In this study, the selected sampling site, Ledbetter Embayment in Kentucky Lake, which is located away from main channel was expected to best represent an accumulation of organic contaminants over time.

PAH trends in sediment cores have been investigated in relatively undisturbed embayment areas that might favor preservation of PAHs (Alexander et al. 1999; Kannan et al. 2005). The differences with other sites in degree of persistence within the cores and suspended sediment may be due to hydrologic setting as well as grain size, geology and climate (Holbrook et al. 2004). Metre and Mahler (2004) have studied PAH trends in sediment cores collected from Lake Como, Echo Lake, and Fosdic Lake in Fort Worth, Texas, as well as Harris Pond and Upper Mystic Lake in Boston, Massachusetts. The total PAHs were found in relatively high concentrations at the tops of cores from Echo Lake, Harris Pond, and Upper Mystic Lake. These profiles are similar to those found in cores collected from Ledbetter Embayment in Kentucky Lake. Additionally, there are differing trends. The PAH profiles showed the increasing concentration and then a decrease in the top section of sediment cores in Lake Como and Fosdic Lake (Metre and Mahler 2004).

Accumulation in Mussel Samples

PAHs were found in all mussel samples collected from the lowermost Tennessee River and Kentucky Lake. This represents accumulation of PAHs in aquatic organisms in the freshwater ecosystems (Page et al. 2004). PAH

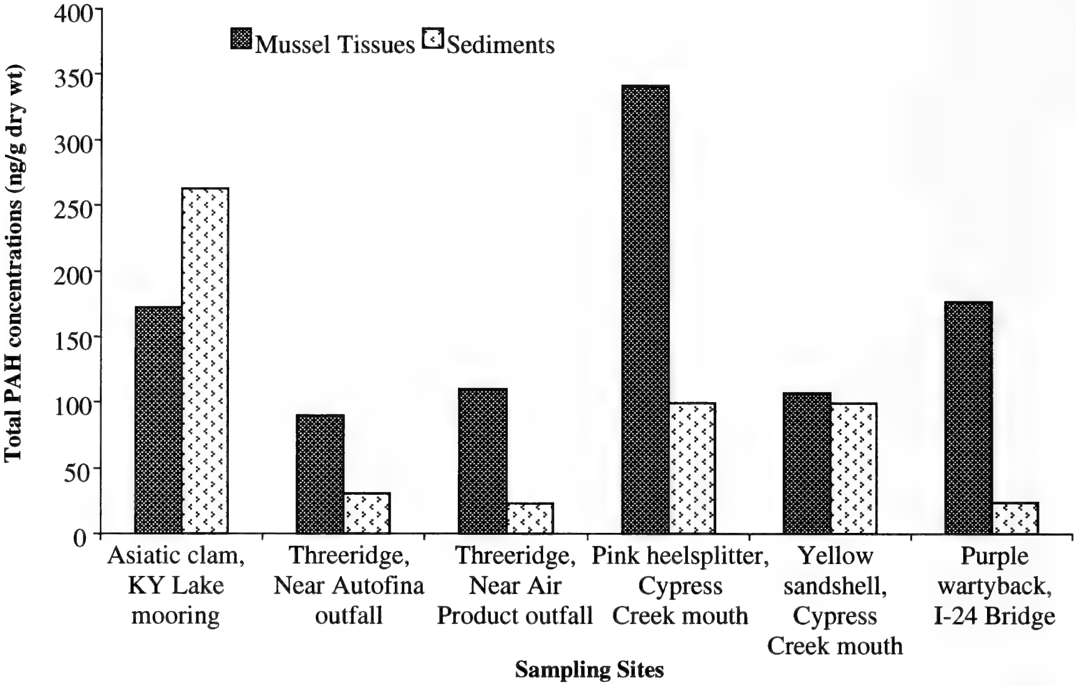

Figure 6. PAH concentrations in mussel tissues in comparison with concentrations in sediments from the same sampling sites.

concentrations found in mussels are lower than the amounts reported by the National Oceanic and Atmospheric Administration (NOAA) Mussel Watch Project. The low molecular weight PAHs reported ranged from not detected or barely detected (detection limits 3.3 to 67 ng/g dry wt) to 4,200 ng/g dry wt. The mean concentrations of high molecular weight PAHs in that project were reported from below detection limit (detection limits 3.9–47 ng/g dry wt) to 11,000 ng/g dry wt (Page et al. 2004).

In our study, naphthalene and phenanthrene were found predominantly in mussel samples from the lowermost Tennessee River and Kentucky Lake. Except for the sampling site at TRM 15.5 near the Atofina outfall and Cypress Creek mouth (TRM 10.1), the predominant PAHs found were phenanthrene, benzo[b]fluoranthene, naphthalene, anthracene and benzo[a]pyrene respectively. Total PAH concentrations ranged from 90.1 to 340.9 ng/g dry wt in a threeridge mussel (*Amblema plicata*) at TRM 15.5 near the Atofina outfall and in a pink heelsplitter (*Potamilus alatus*) at the mouth of Cypress Creek (TRM 10.1).

Comparing PAH concentrations in mussel samples with PAHs measured in sediment from the same sampling site, there was no clear relationship (Figure 6). Due to high deposition of suspended sediment at the mouth of Cypress Creek (TRM 10.1) and the mooring site (TRM 23.1) in Kentucky Lake, the highest total concentration of PAHs were found in pink heelsplitter at the mouth of Cypress Creek (TRM 10.1) and in relatively high concentrations in the Asiatic clam (*Corbicula. fluminea*, age 5 years) at the mooring site (TRM 23.1) in Kentucky Lake. Because the highest amount of total fat was found in the Asiatic clam at the mooring site in Kentucky Lake, total PAHs were found in relatively high concentration with the youngest age of collected mussel samples. The low molecular weight PAHs are the predominant species found in the mussels.

Nakata et al. (2003) reported total PAH concentration in biota such as clams, oysters, lugworms, crabs, mudskippers (herbivore), mudskippers (omnivore), and other omnivore fish collected from a tidal flat in the Ariake Sea, Japan. The highest PAH concentrations were reported in lugworms (24 ± 2.5 ng/g wet

wt.), followed by oysters (6.3 ± 1.8 ng/g), clams (6.3 ± 3.0 ng/g), crabs (4.2 ± 1.4 ng/g), herbivore mudskippers (2.9 ± 1.5 ng/g), omnivore mudskippers (0.37 ng/g), and other omnivore fishes (0.37 ng/g). The lowermost Tennessee River and Kentucky Lake samples showed relatively higher accumulations compared with the amounts reported in the biological samples from marine environment (Nakata et al. 2003). Several authors reported that environmental (soil, sediment) and biological samples (freshwater mussels, fish) from freshwater environment contain higher PAHs concentration than marine environments (Metre and Mahler 2004; Yan et al. 2005). Differences in the PAHs contamination level may attributable to sources of PAHs and distance from the source.

CONCLUSIONS

Polynuclear aromatic hydrocarbon (PAHs) measurements made in sediments and mussel tissues collected from the lowermost Tennessee River and Kentucky Lake revealed several findings with respect to chemical characteristics governing the distribution and bioaccumulation of these pollutants. Based on limited number of samples analyzed and 7 target PAHs, the following observations were made:

- PAH concentrations were detected in all sediments and mussel tissues.
- Sediment collected from the mooring site (TRM 23.1) in Kentucky Lake was recorded the greatest concentrations of total PAHs. Greater deposition of suspended sediment due to relatively less water current at this site and higher barge traffic might have contributed to relatively higher concentrations of PAHs. Phenanthrene, benzo[b]fluoranthene, naphthalene were predominantly found at this sampling site.
- The lowermost Tennessee River and Kentucky Lake are comparatively less polluted with total PAH compounds than other selected freshwater ecosystems in the United States.
- PAH profiles showed preferential accumulation of low molecular weight PAHs (naphthalene and phenanthrene) in the deeper cores. The profiles of sediment cores suggested that PAHs were relatively stable in deeper section of the core sediments.
- Accumulation pattern of PAH species in sediment and mussel tissues exhibited the following order: naphthalene > phenanthrene > benz[a]pyrene > anthracene > benzo[b]fluoranthene > benzo[k]fluoranthene > benzo[g,h,i]perylene. The low molecular weight PAHs were predominant species accumulated in mussels at all sampling sites.
- Several PAHs have been considered human carcinogens that can be classified into two categories depending on their properties: carcinogenic and non-carcinogenic PAHs. The composition of PAHs in sediments from the lowermost Tennessee River and Kentucky Lake revealed that 66.2% (w/w) of the total PAHs were noncarcinogenic PAHs, and 33.8% (w/w) were carcinogenic PAHs.

The present study provides evidence of the spatial, temporal distribution and bioaccumulation of selected PAH compounds in sediments and freshwater mussels from the lowermost Tennessee River and Kentucky Lake. Future monitoring studies including more number of samples and 16 priority PAHs are essential to identify the sources of contamination, and effects on environment and organisms.

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J. Ky. Acad. Sci. 68(2):199–201. 2007.

INDEX TO VOLUME 68

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J. Ky. Acad. Sci. 68(2):202–204. 2007.

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